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CONTROL OF SEXUALLY TRANSMITTED INFECTIONS IN A HIGH PREVALENCE REGION: HIV, SYPHILIS AND HBV AMONG YOUNG ADULTS AND PREPARATION FOR HIV VACCINE TRIALS IN MOZAMBIQUE

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Control of Sexually Transmitted Infections in a High Prevalence Region: HIV, Syphilis and HBV among Young Adults and Preparation for HIV Vaccine Trials in Mozambique

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Dedication

To my family.

ABSTRACT

Mozambique is one of the countries with a high burden of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and syphilis. HIV seroprevalence in adults was estimated to be 11.5% in 2009, syphilis prevalence among women was estimated to be 5.7% in 2010 and an estimated national HBV prevalence of above 8%. These infections are sexually transmitted and co-infections are common. The availability of data on prevalence and incidence of these infections, and risk factors related to their spread, is crucial for designing prevention strategies and in order to successfully conduct clinical intervention studies. Additionally, a safe, efficacious and affordable HIV vaccine would be ideal to better control the HIV epidemic.

In study I, we prospectively enrolled (n=1380) and followed up a cohort of young adults (n=1309, HIV seronegative participants) to determine their suitability for possible participation in phase I/II HIV vaccine trials through determination of prevalence and incidence of HIV. We also determined the prevalence of HBV and syphilis. The incidence of HIV in this group was 1.14/100PY. The prevalence of HIV, HBV and syphilis was 5.1%, 12.2% and 0.36%, respectively. **In study II**, we determined the prevalence of HBV seromarkers in 1377 young adults in Maputo, Mozambique. The age-specific changes of prevalence of HBsAg and Anti-HBc was used to estimate the incidence of HBV using catalytic modelling. The overall prevalence was 42.8% for exposure to HBV (anti-HBc+), 12.1% for HBV infection (HBsAg+), 8.5% for chronic carriers (HBsAg+ and anti-HBc+) and 3.6% for acute infection (HBsAg+ only). The incidence of HBV was 180 per 100,000 PY using HBsAg and 2690 per 100,000 PY using anti-HBc. **In study III**, a cross sectional study was conducted at a youth clinic in Maputo, Mozambique, to establish, for the first time, clinical laboratory reference values in the country. The hematological and biochemistry reference values derived from 257 healthy Mozambican young adults differed from those derived from a North American population. **In study IV**, a phase I trial was conducted to assess the safety and immunogenicity of HIV-DNA delivery using the Zetajet ™, a needle-free device, in a volume of 0.2 ml (3 mg/ml) intradermally followed by HIV-MVA boosts. The volunteers were randomized to receive three immunizations of 600 μg (2 x 0.1 ml, standard injection) (n = 10) or 1200 μg (2 x 0.2 ml) (n = 10) of HIV-DNA, followed by two 10^8 pfu HIV-MVA boost vaccinations. Four subjects received placebo. After the first HIV-MVA, Env responses were significantly higher in the high-dose group compared to the low-dose group (median 420 vs. 157.5 SFC/million PBMC, p = 0.014). Preliminary data show that the frequency of responders with antibodies against HIV Env antigens in the V2 loop was significantly higher in the high-dose group than in the low-dose group, 6/8 (75%) vs. 2/8 (25%), respectively, p = 0.0486. The high dose of HIV-DNA induced the higher average number of Env-reactive antigen features in the V2 loop than the low dose.

Conclusion: The prevalence and incidence of HIV in the youth cohort in Maputo was relatively low, suggesting that this group is suitable for recruitment into a phase I/II HIV vaccine trial. The prevalence and incidence of HBV was high among young adults in Mozambique. Therefore, further HBV prevention strategies should be implemented, including catch-up vaccinations for children and adolescents, screening of pregnant women and vaccination of adults at risk of exposure. The clinical laboratory reference values established here highlight the need for establishing region-specific values for proper patient management and the safe conduct of clinical trials in Mozambique. The last study demonstrated that priming with a high dose of HIV-DNA is safe and suggests an immunological advantage over the lower HIV-DNA dose.

LIST OF SCIENTIFIC PAPERS

I. Viegas EO*, **Tembe N***, Macovela E, Gonçalves E, Augusto O, Ismael N, Sitoe N, De Schacht C, Bhatt N, Meggi B, Araujo C. 2015. Incidence of HIV and prevalence of HIV, Hepatitis B and syphilis among youths in Maputo, Mozambique: a cohort study. PloS one, 10(3): e0121452.

II. **Tembe N**, Augusto O, Maueia C, Cumbane V, Viegas E, Osman N, Andersson S, Nilsson C, Jani I. A high prevalence of hepatitis B virus among unvaccinated young adults in Maputo, Mozambique indicates a potential transmission risk. *Manuscript submitted*

III. **Tembe N**, Joaquim O, Alfai E, Sitoe N, Viegas E, Macovela E, Gonçalves E, Osman N, Andersson S, Jani I, Nilsson C. 2014. Reference values for clinical laboratory parameters in young adults in Maputo, Mozambique. PloS one, 9(5): e97391.

IV. Viegas EO*, **Tembe N***, Nilsson C, Meggi B, Maueia C, Augusto O, Stout R, Scarlatti G, Ferrari G, Earl P, Warhen B, Andersson S, Robb M, Biberfeld G, Jani I, Sandström E and the TaMoVac study group. Intradermal HIV-1 DNA immunization using needle-free Zetajet™ injection followed by HIV-modified vaccinia virus Ankara vaccination is safe and highly immunogenic in Mozambican young adults: a phase I randomized trial. *Manuscript*

*Authors contributed equally to the work.

CONTENTS

LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine transaminase
Anti-HBc	Hepatitis B core antibody
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
CBC	Complete blood count
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CMDR	Chiang Mai double recombinant
CRF	Circulating recombinant forms
DAIDS	Division of AIDS
DFA-TP	Direct fluorescent antibody test for *Treponema Pallidum*
DFM-TP	Dark field microscopic for *Treponema Pallidum*
DNA	Deoxyribonucleic Acid
EDCTP	European & Developing Countries Clinical Trials Partnership
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FTA-ABS	Fluorescent treponemal antibody-absorption
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HDL	High-density lipoprotein

HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV	Herpes simplex virus
Ig	Immunoglobulin
INS	Instituto Nacional de Saúde
INSIDA	Inquérito Nacional de Prevalência, Riscos Comportamentais e Informação sobre o HIV e SIDA em Moçambique
IPs	Investigational products
IR	Incidence rate
IRR	Incidence rate ratio
MFI	Mean fluorescent intensity
MVA	Modified vaccinia Ankara
Nab	Neutralizing antibodies
NIH	National Institutes of Health
PCR	Polymerase chain reaction
PR	Prevalence ratio
RNA	Ribonucleic acid
RPR	Rapid plasma reagin
Sida	Swedish International Development Agency
SIV	Simian immunodeficiency virus
SSA	Sub-Saharan Africa
STI	Sexually transmitted infection
STS	Serologic tests for syphilis
TPHA	Treponema pallidum hemagglutination assay
TPPA	Treponema pallidum particle agglutination
VDRL	Venereal disease research laboratory
WHO	World Health Organization

1. INTRODUCTION

1.1. Sexually Transmitted Infections: HIV, syphilis And hepatitis B

Today, more than thirty pathogens are known to be sexually transmitted. Among them, eight are responsible for the majority of the incidence of sexually transmitted infections (STIs). Of these eight, four are curable; these are syphilis, gonorrhoea, chlamydia and trichomoniasis. Another four are viral infections and are incurable, namely hepatitis B, herpes simplex virus (HSV), human immunodeficiency virus (HIV), and human papillomavirus (HPV) (1).

According to WHO, approximately 1 million people acquired STIs every day worldwide (1). Sub-Saharan Africa (SSA) is the epicentre of the HIV epidemic (2), and other STIs are still endemic in this region of the world (3). STIs have been associated with many health problems, which include cervical cancer, pelvic inflammatory disease, infertility, fetal wastage, ectopic pregnancy, and related infant and maternal mortality (4). In addition, STIs such as syphilis and herpes simplex type-2 facilitate HIV acquisition (5; 6). Furthermore, HIV has an impact on the disease course caused by syphilis (5) and HBV (7), accelerating their progression and making them more aggressive.

In general, STIs are preventable but there are a substantial number of new cases occurring worldwide (1). This underscores the need of further improvement of the control of STIs. With regard to HIV, there is a clear need of a prophylactic vaccine that can prevent the spread of HIV globally and particularly in SSA, where approximately 70% of all HIV infected individuals are found. The available methods to prevent HIV infection are dependent on the awareness and adherence, which can affect their efficacy (8; 9; 10). HBV infection can be prevented by vaccination, but it continues to be a serious health problem, especially in SSA (11), where technical, logistical, political and social factors represent a challenge for immunization programs (12).

In many African countries there is a lack of data on prevalence and incidence of STIs. The availability of data on the epidemiology of STIs, and risk factors related to their spread is crucial for designing prevention strategies and in order to successfully conduct clinical intervention studies. It is therefore important to make epidemiology data available, and to develop and evaluate vaccines, particularly for HIV, to improve the control of these infections in a high prevalence setting.

1.2. HIV/AIDS

1.2.1. Epidemiology: Global situation

According to WHO, in 2015 approximately 36.7 million (0.8%) people globally were living with HIV, 2.1 million people became newly infected and 1.1 million people died from HIV-related causes. SSA continues to be the region of the world most affected by HIV, where 25.6 million people were living with HIV in 2015. SSA was responsible for 70% of the new infections that occurred in 2015 (2).

Globally, the rates of new HIV-1 infections and of deaths related to acquired immune deficiency syndrome (AIDS) have declined between 2000 and 2015, by 35% and 28%, respectively, due to increased availability of antiretroviral therapy (ART). By the end of 2015, 17 million people living with HIV were receiving ART (2), up from 13.6 million in June 2014. In SSA, the HIV-1 infection rate fell by 41% between 2000 and 2014. The number of deaths related to AIDS in SSA declined by 48% between 2004 and 2014. Within the same region, the number of people receiving ART increased from 100 000 in 2002 to 10.7 million in 2014 (13).

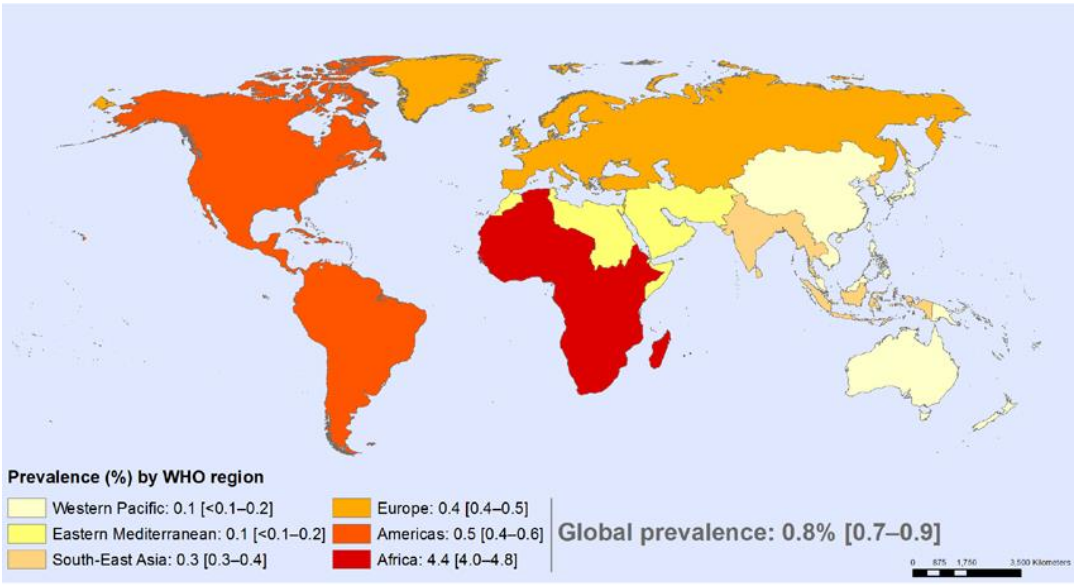

Figure 1. HIV prevalence in 2015, among adults aged 15-49 years (14).

1.2.2. Epidemiology: HIV/AIDS in Mozambique

Mozambique has one of the world's highest HIV prevalences (figure 1). Data from the national community-based survey (INSIDA) indicated that in 2009, 11.5% of adults aged 15-49 were living with HIV in Mozambique, with HIV prevalence being higher among women than men

(13.1% vs. 9.2%). For both women and men, the prevalence increases with age until it peaks. For women the peak occurs between the ages 25-29 and for men between the ages 35-39. Among children aged 0 - 11 years, HIV prevalence is estimated to be 1.4%. Regional prevalence varies substantially, estimates range from 3.7% - 9.4% in the northern region, 7% - 15.5% in the central area and 8.6% - 25.1% in southern region of the country (15).

The first case of HIV in Mozambique was reported in 1986 (16). By 2004, the prevalence of HIV among pregnant women was 16%. In 2007, the prevalence was reported to be 13.2%. From 2009 to 2011, HIV prevalence increased from 13.7% to 15.8% (figure 2). It is possible that the increased availability of ART may have contributed to reduction of deaths related to AIDS and resulted in an increasing HIV prevalence. Overall, HIV prevalence among pregnant women has stabilized in Mozambique, oscillating between 13% and 16% during the period from 2002 to 2011 (17).

The rate of new HIV-1 infections in the country declined between 2001 and 2013, from 160 000 people to 120 000 people, respectively (18). ART may have also contributed to the reduction of HIV incidence in the country.

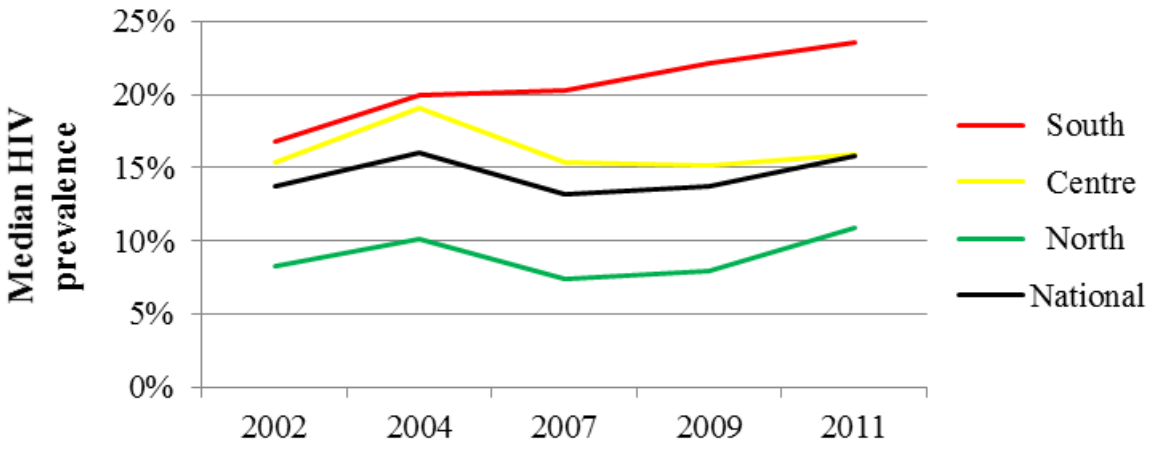

Figure 2. Trend of the median overall and regional HIV prevalence in Mozambique, from 2002 to 2011 (17).

1.3. HIV and AIDS

AIDS is a widespread disease caused by HIV. HIV infects and destroys a variety of human cells. The consequence of severe immunologic damage is AIDS, which is characterized by the loss of an effective immune response to specific opportunistic pathogens and tumours (6).

HIV is part of the Lentivirus family, and it is divided into two types, namely HIV-1 and HIV-2. HIV-1 is the most common and pathogenic strain of the virus. HIV-2 causes AIDS with slow disease progression and it is largely confined to West Africa. HIV-1 is classified into three sub-groups: Group M (major), Group O (outlier), and Group N (non-M or O). Groups O and N are restricted to West Africa. Group M, which is the cause of the global HIV pandemic, consists of nine subtypes: A–D, F–H, J, and K, and many circulating recombinant forms (CRFs) (6). Subtypes A and F can be further divided into sub-subtypes: A1 to A2 and F1 to F2, respectively. Subtypes A, B, and C are the most prevalent HIV-1 genetic forms. Subtype C is responsible for about 50% of the HIV-1 infections worldwide and it is predominate in southern Africa and India. Subtype A is predominant in central and eastern Africa, and in Eastern European countries, while subtype B is most frequent in western and central Europe, the Americas, and Australia, and in many countries of Southeast Asia, northern Africa, and the Middle East. The CRFs account for 18% of the HIV infections worldwide, CRF01_AE (previously called subtype E) is predominant in Southeast Asia, while CRF02_AG is predominant in West and West Central Africa (19). In Mozambique, HIV subtype C is the predominant subtype (20). The enormous genetic diversity of HIV-1 may have implications for differential rates of disease progression, emergence of ART resistance, and the development of an effective HIV-1 preventive vaccine with broad efficacy against all subtypes (19).

1.3.1. Viral structure and replication cycle of HIV

HIV is a spherical particle with a diameter of approximately 100-120 nm. The virus comprises of an envelope that surrounds the nucleocapsid (core), which contains the HIV genome and enzymes (reverse transcriptase, protease and integrase). The viral envelope consists of a lipid bilayer acquired from the host cell membrane after viral budding and viral glycoproteins (gp) (gp120 and gp41). The gp120 is responsible for the attachment of the virus to the host cell and the gp41 mediates the cell fusion process. Between the envelope and nucleocapsid lies the p17 matrix protein. The nucleocapsid is composed of the viral protein p24, typical of lentiviruses (Figure 3). The HIV genome consists of two identical 9kb single stranded RNA molecules,

which contains nine genes. Among the nine genes, three are most important for the viral replication, namely gag, env and pol. The gag gene encodes proteins of the nucleocapsid, matrix and nucleo-proteins. The env gene encodes glycoproteins of the envelope, and the pol gene encodes three viral enzymes, namely reverse transcriptase, protease and integrase (21). The genome includes six accessory genes, which are: tat, rev, nef, vif, vpr and vpu (for HIV-1) or vpx (for HIV-2) (22).

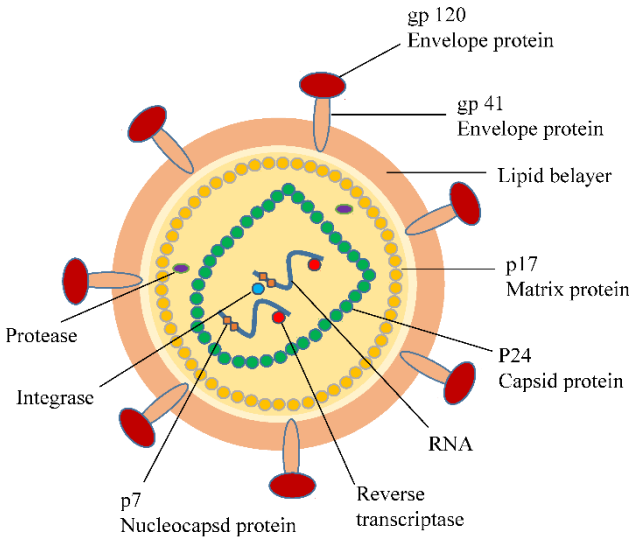

Figure 3. Structure of HIV mature virion particles.

HIV has an affinity to cells expressing CD4 molecules, such as, T helper lymphocytes, monocytes and macrophages, and dendritic cells. (1) The viral gp120 molecules bind tightly to CD4 ligand on the host cells resulting in a conformational change in the gp120, exposing the chemokine binding domains of gp120 and allowing them to interact with the co-receptor CXCR4 or CCR5 on the host cell. Upon interaction between the gp120 and the host chemokine receptor, fusion takes place induced by the gp41 protein. (2) After the fusion, the HIV RNA and various enzymes are released into the cell, including reverse transcriptase. The reverse transcriptase commands the transcription of the single-stranded viral RNA genome into a double-stranded HIV DNA, which is transported into the cell nucleus. (3) The enzyme integrase is responsible for the integration of the proviral HIV DNA into the host's chromosomal DNA. (4) The provirus is transcribed by the host RNA polymerase, resulting in several mRNAs and genomic RNA. (5) The mRNAs are translated into viral proteins, which are cleaved by the HIV-1 protease enzyme. (6) These viral proteins and the replicated viral genomic RNA are

assembled and then buds through the cell membrane, producing a mature infectious virus (Figure 4) (21).

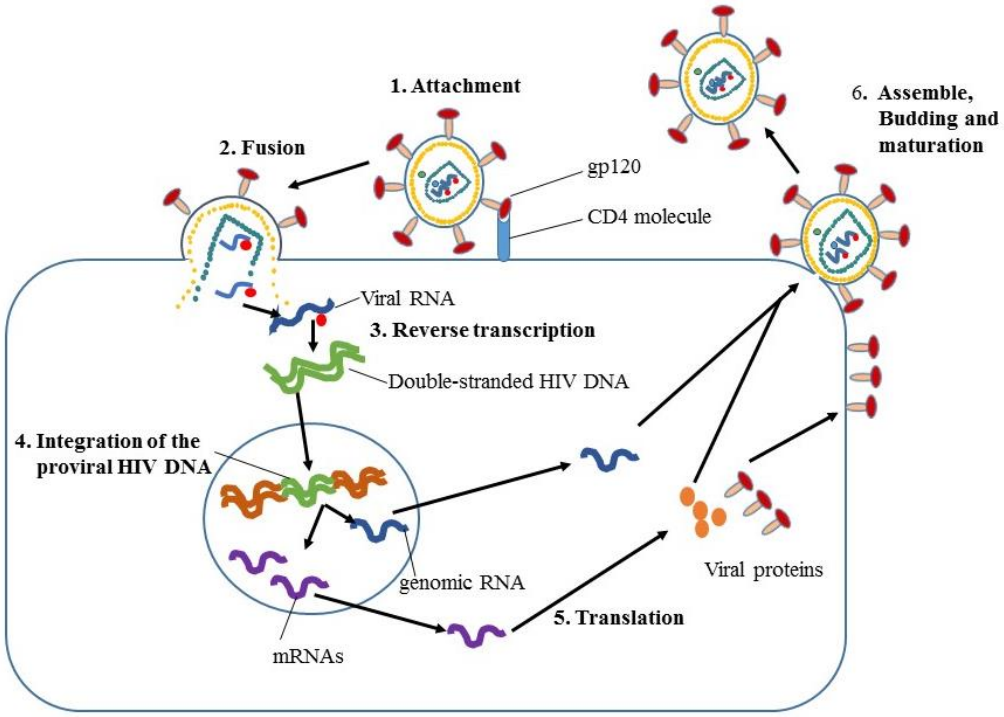

Figure 4. HIV life cycle.

1.3.2. Mode of HIV transmission

HIV, the cause of the AIDS pandemic, is a result of multiple cross-species transmissions of simian immunodeficiency viruses that naturally infect African primates (23). HIV spreads through sexual contact, exposure to infected blood and its products, intravenous drug use and vertically from mother-to-child (during pregnancy, delivery, or breastfeeding) (21). Sexual contact is the most common mode of HIV transmission. Factors, such as high HIV-1 viral load and high-risk sexual behaviour including the presence of STIs, having many sexual partners and unprotected sex (not using a condom) increase the risk of HIV sexual transmission. Mother-to-child HIV-1 transmission also plays an important role in the HIV epidemic. In the absence of interventions, HIV-1 vertical transmission is estimated to occur at a rate of 25% during delivery and 8.9/100 child-years of breastfeeding after the fourth week, with higher rates during the first four weeks (6; 21). Sharing unsterilized injection equipment and accidental needlestick injuries can be another source of HIV infection (21). An increased risk of HIV-1 transmission by stick injuries or vertical mode is also associated with high level of HIV-1 viral load in the infected individual (6).

In Mozambique, heterosexual transmission of HIV is responsible for the majority of new HIV infections in adults. Factors, such as multiple sexual partners, low rate of condom use, mobility and migration, sexual intercourse between people of different generations and transactional relationships, and low rate of male circumcision drive the epidemic in the country (24).

1.3.3. Diagnosis of HIV infection

HIV antibody tests are the most commonly used for the diagnosis of HIV infection. However, these tests may give false negative results during the window period (between the time of infection and production of detectable level of antibodies). Antibodies are usually detectable around 3-4 weeks after the infection. HIV testing is usually performed in two phases. First, a high sensitivity screening test is performed, and if the outcome is positive a second test with high specificity is performed to confirm the result. The enzyme-linked immunosorbent assay (ELISA) is the most commonly used for screening purposes and the Western blot procedure as a confirmatory test. However, the main disadvantage of the WB assay is related to the high cost and the need of skilled people to perform the test. Later generations of ELISAs are able to detect the presence of both the antibodies and the viral p24 antigen. These ELISAs reduced the window period to almost the levels of the detection of virus RNA, which is about 10-12 days after infection (25). Some of the antibody/antigen tests are designed to be rapid HIV tests that produce results within 15-30 minutes. The advantage of this test is that they have similar accuracy rates to traditional blood tests, while requiring fewer logistics (26). Other methods used for HIV diagnosis include tests that detect the presence of the virus by HIV nucleic acid detection, using polymerase chain reaction (PCR) and p24 antigen testing. These tests are able to detect early HIV infection before seroconversion. The p24 antigen test detects the viral p24 antigen in the serum of a HIV infected patient. PCR tests are limitedly used due to the costs and the need of skilled staff to perform the test. The qualitative DNA PCR, which allows the detection of viral DNA integrated into the host cell's genomic DNA, is particularly useful for testing infants younger than 18 months of age, born from HIV-positive mothers, since HIV antibody assays may detect maternal antibodies that these infants carry. Quantitative RNA PCR (viral load), which detect plasma viral RNA, is recommended to monitor HIV-positive individuals before or during antiretroviral therapy (27).

The Mozambican national algorithm for HIV testing for adults and infants older than 18 months is comprised by two rapid tests, the Determine HIV-1/2 (Inverness Medical, Bedford, United Kingdom) as the screening test, followed by the UniGold HIV-1/2 (Trinity Biotech, Bray, Ireland)

as the confirmatory test. For individuals with indeterminate results, defined as a reactive Determine test followed by a non-reactive UniGold assay, the algorithm is repeated immediately. If the result continues to be indeterminate, HIV testing is repeated after 3-4 weeks and if the same result is still produced, the algorithm is repeated after 6-8 weeks. Only then, if the result still is indeterminate is a venous blood sample collected and sent to the reference laboratory (28) where the algorithm is repeated and discordant results are resolved by using PCR.

1.3.4. Prevention of HIV infection

There are several methods to prevent HIV infections, namely education on HIV, condom use, counselling and testing for HIV, ART, pre- and post- exposure prophylaxis, screening of blood and its products, prevention and treatment of STIs and medical male circumcision (6). A HIV vaccine is not currently available as it is still under development. The combination of the available methods has contributed to the reduction of HIV incidence in most countries, but the epidemic still not under control and the virus continues to spread at high rates. This could be because most of the available methods are dependent on awareness and adherence (29). Education plays an important role in the prevention of HIV infection. Reviews of education on HIV are unanimous, in that all programmes targeted at an increase in knowledge about HIV/AIDS, contribute directly or indirectly to behaviour change and consequently reduce the risk of infections (30). Condom use is essential to prevent HIV transmission (31) and other sexually transmitted infections. The perception that condoms reduce sexual pleasure, increase infidelity, that some have an unpleasant smell, and its limited availability have been reported to affect its use (32). Counselling and testing for HIV have significant benefits. Knowing the HIV status of individuals who are positive can result in referral to ART in order to lower the viral load, thereby prolonging life and heath, and reducing the risk of HIV transmission to others by 96%. Furthermore, for individuals who have negative results, this information can help to reduce high-risk behaviour and continue good health (33). ART has also been used for pre- and post-exposure prophylaxis and for prevention of mother-to-child HIV-1 transmission (6). Screening of blood and its products is essential in order to exclude blood donations at risk of transmitting infection from donors to recipients (34). Treating co-infections, such syphilis (5) and herpes simplex type-2 infection, reduce the HIV viral load and transmission. Male circumcision is associated with a significantly reduced risk of HIV infection and can be used as an additional HIV prevention strategy (6).

Mozambique has defined strategies and plans for HIV prevention, which includes all the methods mentioned above (24). With these programmes and plans, Mozambique has achieved significant progress in the prevention of HIV infections but additional efforts should be made in order to control the spread of HIV in the country.

Despite increasing knowledge about HIV and methods to prevent infections among the Mozambican population, the behaviour in relation to HIV has not changed significantly. The INSIDA 2009 survey showed that 67% of females and 76% of males age 15-49 knew of condom use as a HIV preventive method. However, only 8% of women and 16% of men used a condom at the last time of sexual intercourse (15). The Demographic and Health Survey 2011 found that 3% of women and 30% of men age 15-49 reported to have had two or more sexual partners in the past 12 months. The rates of HIV testing are still very low in the country, and only 45% of women and 23% of men age 15-49 have ever been tested for HIV. The levels of stigma and discrimination against people infected with HIV are still high in the country, but there is a decreasing trend. Additionally, the rate of circumcised men is still low in Mozambique, at around 47% among men age 15-49 (35).

ART was introduced in Mozambique in 2003/2004 (24). Subsequently, the number of people in antiretroviral treatment increased substantively and by 2012 the coverage among eligible patients to ART in country reached 45% (36). Post-exposure prophylaxis is provided for post-occupational exposure to HIV and for rape victims (37).

The prevention of mother-to-child transmission program was fully introduced in Mozambique in 2006 (38), and as result of the program the number of new infections among children declined by 50% or more between 2009 and 2013 (39).

The HIV Early Infant Diagnosis program, which provides HIV testing for infants younger than 18 months of age using DNA PCR, was introduced in the country in 2006. This program is contributing to the reduction of AIDS related deaths among children by improving the testing rates, which result in the early initiation of ART.

Finally, specific interventions targeting key populations such as men who have sex with men (MSM), commercial sex workers (CSW), miners, truck drivers, and others are being designed.

1.4. HIV vaccine

The development of a safe and effective HIV-1 vaccine is critically important to control the spread of the virus, since the efficacy of the available methods is dependent on adherence. However, despite the urgent need, a HIV vaccine still not available. The reasons why include the genetic diversity of the virus, the capacity of the virus to evade adaptive immune responses, the early establishment of latent viral reservoirs, the difficulty in the development of antigens that are able to induce broadly reactive antibody responses, uncertainty about what constitutes protective immunity (40) and the absence of appropriate animal models (41).

Several progressions in the development of a HIV vaccine have been made. Studies in animals have demonstrated that vaccine regimens expressing the simian immunodeficiency virus (SIV) Gag, Pol and Env antigens conferred protection against acquisition of SIV infection and that Env was crucial for the protective effect of the vaccine (42; 43). Animal models have also suggested that Env-specific antibodies are essential for blocking virus acquisition (43) and Gag-specific cellular immune response for the control of viremia (42) in the vaccinated monkeys. Unfortunately, the vaccine designs used in pre-clinical trials, which had shown efficacy, did not produce the same result in humans (44; 45). However, they have contributed valuable information for the development of a HIV vaccine. Studies on humans indicate that T cytotoxic cells ($CD8^+$ T cells) and $CD4^+$ T cells can mediate control of viral replication in the HIV infected individual (46; 47). The results from animal models and studies on humans indicate that both humoral and cell-mediated HIV-specific immune responses would be important protective factors against HIV infection. Based on these suggestions, the majority of vaccine candidates that are being produced for use in clinical trials, aim to induce both antibody-mediated and cell-mediated HIV-specific immune responses. Despite more than 180 clinical trials conducted, HIV-1 vaccine development efforts, to have a prophylactic or therapeutic vaccine, have not yet proven successful. Six HIV vaccine efficacy trials (phase IIb and III) have been conducted but only one, the RV144 phase III trial in Thailand, showed protective efficacy, about 60% at 12 months (48) and 31% at 42 months, after completion of the vaccine series (49). The results from this trial are guiding the research on HIV vaccine today.

1.4.1. HIV-1 Vaccine Strategies

Viral infections have been successfully controlled by vaccines based on the live attenuated viruses, whole killed viruses and protein subunits. In the search for a HIV vaccine, live attenuated virus vaccines have been tested in the SIV/macaque model and have showed substantial protective efficacy against SIV challenges in rhesus monkeys. However, these approaches are not being considered for HIV-1 due to significant safety concerns. The utility of whole killed viruses and protein subunits is limited, due to their inability to induce broadly reactive neutralizing antibody (NAb) responses and by their inability to elicit CD8+ T lymphocyte responses. For HIV-1, novel vaccine strategies are being used. These strategies include gene delivery technologies, such as plasmid DNA vaccines and live recombinant vectors that are engineered to express HIV-1 antigens (40). DNA vaccines have been shown to induce predominantly T cell responses that were able to control viral replication and delay T CD4+ cells decline in vaccinated macaques challenged with SIV (50; 51; 52). Multiple injections of high doses of DNA vaccines are typically required to elicit detectable immune responses in nonhuman primates and humans. Therefore, research continues on the development of adjuvants for DNA vaccines and improved delivery technologies, to improve DNA immunogenicity (40). Adjuvants such as IL-12, IL-15 and granulocyte macrophage colony-stimulating factor (GM-CSF) (53), and methods such as in vivo electroporation (40) and needle-free injection devices to deliver DNA vaccine have been shown to enhance the immunogenicity (54). Live recombinant vectors are used alone or in context of boosting the responses induced by DNA vaccines. The most commonly used viral vectors are adenoviruses and poxviruses (40). Prime-boost vaccination regimen using DNA priming and recombinant virus based vaccines, such as recombinant modified vaccinia virus Ankara (MVA) for boosting, has been shown to induce broad and strong HIV-specific T cell responses (54; 55) and also elicited HIV-specific binding antibodies (56) and functional antibodies (57). Live recombinant virus prime-protein boost, or DNA prime-protein boost are other HIV vaccine strategies used (58). The RV144 trial used a recombinant avipoxvirus vaccine boosted with HIV Env protein (49).

1.4.2. Lessons learnt from HIV vaccine phase IIb and III trials

Four HIV-vaccine candidates were tested in six efficacy trials. The first two efficacy trials (phase III) Vax003 and Vax004 used bivalent vaccines containing the monomeric gp120 alone from subtype B/E and subtype B, respectively, and aimed to induce humoral responses. The vaccine failed to prevent HIV-1 acquisition infection or delay disease progression. The vaccine generated higher level of NAb against an easily neutralizable virus (HIV-1 MN) and this was correlated with lower risk of HIV infection. Lower levels of NAb were induced against viruses that were difficult to neutralize and this was not correlated with protection, suggesting that the titers and breadth of response were not sufficient. The HVTN 502 phase IIb (Step) and HVTN 503 phase III (Phambili) trials tested the Merck recombinant Ad5 gag/pol/nef subtype B and aimed to induce specific cell-mediated immune responses. In both studies, the vaccine was able to generate T cell immune responses, but did not prevent HIV-1 infection and there was increased risk of HIV acquisition in vaccinees compared to placebo recipients. In the Step trial, the risk of HIV-1 infection was significantly lower in men with no pre-existent immunity to Ad5 and who were circumcised (59; 60). The Thai RV144 phase III clinical trial tested a recombinant avipoxvirus vaccine boosted with HIV Env protein, aimed to induce both cellular and humoral immune responses. The trial showed a modest efficacy of 31.2%; although the vaccination did not have effect on the HIV viral load and T CD4 count in the infected subjects (49). In this trial binding IgG antibodies to variable regions 1 and 2 of HIV-1 envelope proteins were inversely correlated with the risk for HIV-1 infection, while HIV-1 Env-specific plasma IgA responses directly correlated with risk (61). The HIV-specific T cell response in the RV144 vaccinees, although modest in frequency compared with humoral immune responses, confirmed HIV gag120 V2 specificity, polyfunctionality and functional cytolytic capacity (62). HVTN 505 (phase IIb) is the sixth trial. This study tested gag/pol/env subtype A/B/C DNA prime boosted with rAd5 vector and also aimed to induce cellular and humoral immune responses. This trial showed no efficacy on HIV acquisition, viremia control and CD4 count (59).

The results of these trials suggest that a HIV vaccine is feasible, but innovation to improve immunogens and vaccine strategies is needed. Additionally, clarification of what constitutes protective immunity is also required.

1.4.3. Challenges in conducting a HIV vaccine clinical trial

For conducting clinical studies, a well-organized and experienced regulatory authority is needed in the country where the study is to be conducted. In developing countries, ethical approval processes are often lengthy or not well defined, and there is significant bureaucracy and a lack of regulatory staff with expertise in reviewing. This considerably delays the start of the trials (63).

Conducting a clinical trial demands qualified human resources, infrastructure (clinic facilities, laboratory facilities and data management facilities), and an appropriate population for the study. Clinic facilities are the places where researchers interact with participants for information exchange, counselling sessions, physical exams, and where the vaccine candidate can be injected. Laboratory facilities for clinical trials can be divided into two categories. One is small and is located near the clinic, so that samples can be collected for processing in order to confirm the eligibility of participants. The second category of laboratory facility is more sophisticated and it is responsible for the immunogenicity testing, for example, performing assays to find antibody and cell-mediated immune responses. In addition, data management facilities are required to record the data of ongoing trials, generate reports on study outcomes and archive documents from the study according to good clinical practice guidelines. It is also important to select the appropriate population for the study. Phase I and II trials provide information on the safety of the candidate vaccines and the ability to induce immune responses. For these early phases of vaccine evaluation, healthy volunteers with low-risk for HIV infections are sought. In phase IIb and III trials where the aim is to see if the vaccine is effective, a high-risk population is involved (64). Understanding the targeted population is important to enable high retention.

Finally, it is indispensable to have local clinical laboratory reference values to assess participant eligibility and safety of the product. The use of improper reference values in clinical trials may result in unnecessary exclusion of eligible participants, which can affect the time period of trial enrolment, due to the large number of participants that must be screened in order to reach the target sample size. A long enrolment period impacts on both workload and study cost. The use of non-local derived values may also contribute to over-reporting of adverse events (AEs) (65). Laboratory abnormalities based on non-indigenous laboratory reference values and medical abnormalities were reported to be the main reasons for exclusion of volunteers from two Kenyan HIV vaccine clinical trials (66). Moreover, studies have suggested that use of the US NIH Division of AIDS (DAIDS) toxicity tables may not be appropriate for African populations (65; 67).

1.5. Syphilis

1.5.1. Epidemiology

Syphilis remains a global health problem and it is to a higher degree affecting developing countries. WHO estimated that there were 10.6 million new syphilis cases in adults worldwide in 2008, of which an estimated 3.4 million cases occurred in Africa, 3.0 million in South-East Asia and 2.8 million in the Americas (68). In the WHO region of the Americas, Latin America and the Caribbean are the most affected (3). In North America and Western Europe the incidence of syphilis is low, 5.3 per 100 000 in 2013 (69) and 4.4 per 100,000 in 2010 (70), respectively. However, increasing rates of syphilis have been reported in North America and in many countries in Europe (69; 70).

For Mozambique, WHO reported a syphilis prevalence of 5.7% among women attending antenatal services in 2010 (71). In different parts of the country, syphilis prevalence among pregnant woman in 2009 was 5.8%, 2.0%, and 1.8% in northern, central and southern regions, respectively (16). Data from WHO indicates a trend of decline in rates of syphilis in Mozambique, from 7.9 % in 2008, 6.9% in 2009 to 5.7% in 2010 but the rate of infections is still high (72).

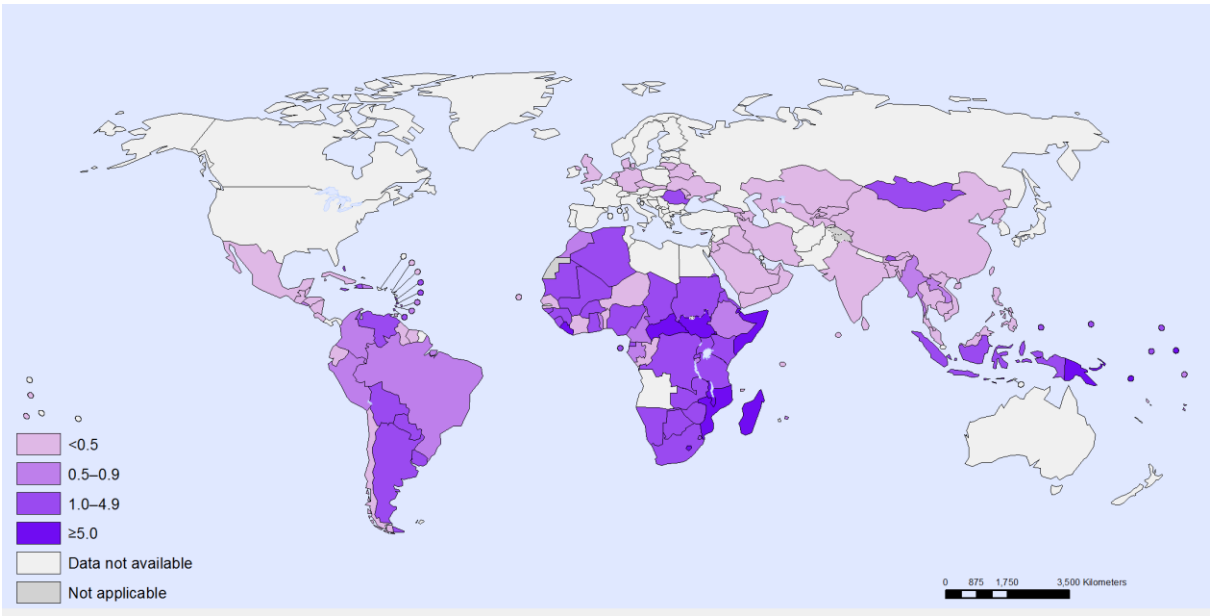

Figure 5. Global prevalence of syphilis among pregnant women (73).

1.5.2. Etiology, Transmission, pathogenesis and clinical features

Syphilis is a sexually transmitted infection caused by the bacteria *Treponema pallidum* subspecies *pallidum. Treponema pallidum* belongs to the order *Spirochaetales*, family *Spirochaetaceae* and genus *Treponema* (74).

Humans are the only known source of treponemal infection. Transmission mainly occurs by sexual contact or through the placenta to the foetus. Syphilis can also be transmitted by kissing, blood transfusion and accidental inoculation. The bacteria can penetrate thought microscopic skin lesions or mucous membranes (74; 75).
The pathogenesis of syphilis appears to be a result of the invasive properties of *Treponema pallidum.* This bacteria has the ability to invade intercellular junctions of endothelial cell monolayers and intact membranes, causing the clinical complications of syphilis (74; 75).

Syphilis has four clinical stages of infection, which are primary, secondary, latent, and tertiary, and may also occur congenitally. The primary syphilis is typically a single chancre, which appears at the site of inoculation, after an incubation period of 3 to 90 days. The primary syphilis disappears spontaneously after few weeks (74). In the secondary syphilis, the most common symptom is a disseminated mucocutaneous rash. Other symptoms may include sore throat, muscle aches, malaise and weight loss. These symptoms usually occur within 12 weeks after initial inoculation and usually resolve spontaneously (75). The period from disappearance of the secondary syphilis until tertiary manifestations appear, which can last for years, is called latent or asymptomatic syphilis. In tertiary syphilis there are gummas (granulomatous lesions), neurological, or cardiovascular symptoms. Congenital Syphilis can cause adverse outcomes in pregnancy, resulting in spontaneous abortion, stillbirth, premature delivery, perinatal death, hydrops fetalis and low birth weight (74).

1.5.3. Clinical course of Syphilis and HIV Co-infection

The clinical manifestations of syphilis and the disease course may be altered in the presence of HIV. Syphilis may be more aggressive and the latency period may be decreased in co-infection with HIV (5). In HIV infected patients, the primary syphilis is more likely to be asymptomatic but it may also consist of multiple chancres as well as large and deeper lesions. HIV infected patients are more likely to present secondary syphilis, which is often more aggressive and

present with atypical clinical manifestations (5; 76). Moreover, patients co-infected with HIV and syphilis are more likely to have neurosyphilis (5; 76; 77) and cardiovascular diseases (77).

Syphilis has an impact on the acquisition of HIV infection. Genital ulcers caused by syphilis increase the risk for HIV acquisition by facilitating the entrance of the virus into systemic circulation and increasing the number of cells receptive to HIV or increasing the expression of cell receptors (5). There is controversy regarding the effect of syphilis on the course of HIV infection. Multiple studies have suggested that syphilis can negatively affect CD4 cell counts and increase HIV replication (78; 79; 80), while others did not find any impact on HIV-1 disease progression (81). Syphilis may have a transient impact on the level of HIV viral load and CD4 count in some patients, which is resolved after treatment of the treponemal infection (82).

1.5.4. Syphilis Diagnosis

Indirect diagnoses (serological test of syphilis - STS) are most commonly used for syphilis diagnosis, regardless of the stage of the disease. However, these tests may be negative in early primary syphilis and the direct identification of the bacteria by dark field microscopic (DFM-TP) or direct immunofluorescent antibody test (DFA-TP) of lesion exudates and tissues, and nucleic acid amplification methods such as polymerase chain reaction (PCR) may be useful to confirm the diagnosis. The limitation of the direct diagnosis of *Treponema pallidum* is basically related to the cost and the need of skilled people to perform the tests (83).

The STS are based on the detection of antibodies and they are divided into non-treponemal and treponemal tests. The nontreponemal tests, such as the Venereal disease research laboratory (VDRL) test and the Rapid Plasma Reagin (RPR) card test, are used as a screening tests and they detect IgM and IgG antibodies against cardiolipin that is present in the sera of patients with syphilis, but can also occur in response to a variety of conditions unrelated to syphilis. The treponemal tests detect antibodies that specifically target the treponema. Treponema-specific antibodies remain even after clearance of infection. Therefore, they are used as confirmatory tests to verify reactivity in nontreponemal tests. Treponemal tests include the fluorescent treponemal antibody-absorbed test (FTA-ABS), Treponema pallidum hemaglutination assay (TPHA), Treponema pallidum particle agglutination (TPPA) and enzyme immunoassay (EIA) (83; 84).

In general, STS are used for screening in both HIV-negative and positive individuals. However, in some cases HIV infection may modify the result of the STS. HIV may cause polyclonal B cell

activation with increased levels of immunoglobulins, which could lead to the positive STS even after elimination of *Treponema pallidum*. HIV infection can also depress B cell functions, which may lead to false negative results in STS. Thus, when clinical findings are suggestive of syphilis and STS are negative, it is recommended to use other tests, such as DFM-TP and DFA-TP using biopsy samples of the lesion, and PCR (5).

1.6. Hepatitis B

1.6.1. Epidemiology

The prevalence of chronic HBV infection varies greatly in the world (Figure 6). There are regions of high, intermediate and low endemicity, as defined below:

High endemicity: regions where ≥ 8% of the population is infected, which include most developing areas, such as South East Asia, China, sub-Saharan Africa and the Amazon Basin.

Intermediate endemicity: regions where 2-7% of the population is infected, which include part of Eastern and Southern Europe, the Middle East, Japan, and part of South America.

Low endemicity: regions where < 2% of the population is infected, which include most developed areas, such as North America, Northern and Western Europe and Australia (85).

According to WHO, 240 million people are chronically infected with HBV and about 686 000 people die annually due to HBV clinical complications (2).

United States of America and Europe are low endemic regions. The USA has an estimation of 0.8–1.4 million of people living with chronic HBV infection, of which 70% were born in countries from high and intermediate endemicity regions (69). In Europe, WHO estimated an overall prevalence of 1.8% HBV carriers among adults in 2013. However, the epidemiology of hepatitis B in the Europe is diverse, the prevalence varies from extremely low (< 0.1% in Hungary) to high (13% in Uzbekistan) (86).

In sub-Saharan Africa, the prevalence of HBV varies from 9-20% (87). For Mozambique, studies have estimated a prevalence of 8.34% to 14.6% in the general population (88; 87), 6.0% to 20.5% in blood donors (89; 90; 91) and 8% among women from a rural area of southern Mozambique (92).

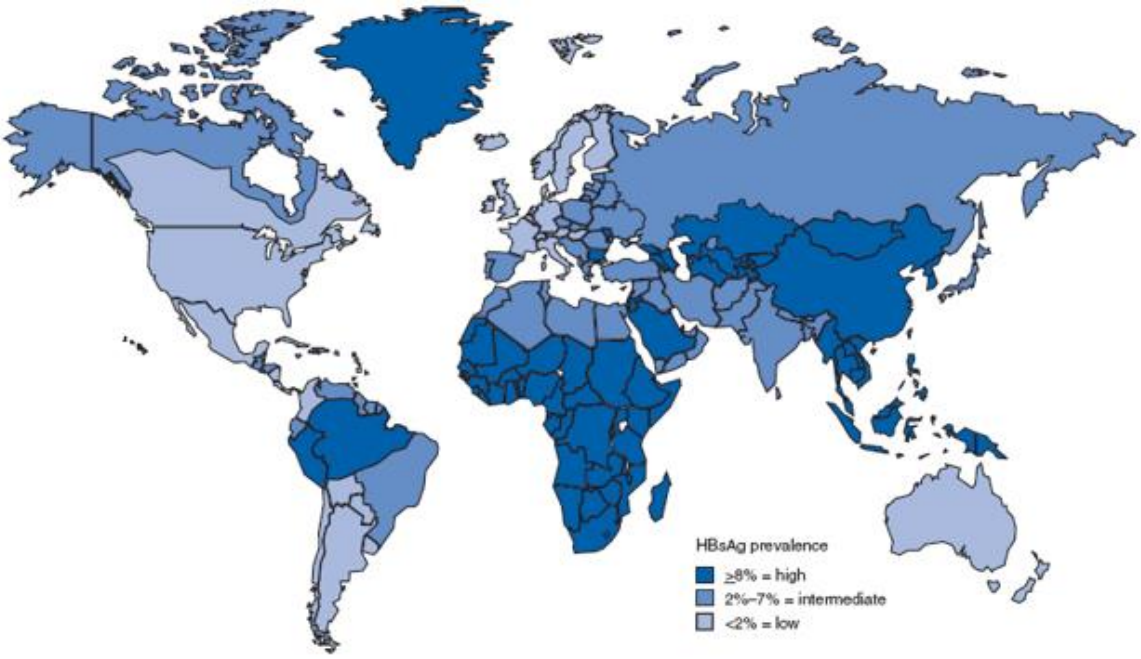

Figure 6: Geographic distribution of chronic hepatitis B infection (93).

1.6.2. HBV and HCC

Only about one-third of adults with acute HBV infection develop clinical symptoms and signs of hepatitis, which include fatigue, nausea, jaundice, and in rare cases, acute liver failure. The clinical incubation period of acute HBV infection ranges from 30–180 days after exposure (94).

Chronic infection with the hepatitis B virus has been associated with hepatocellular carcinoma (HCC) development. In Africa and East Asia, 60% of HCC has been attributed to hepatitis B and in the developed western world; approximately 20% of cases can be attributed to HBV infection (95). The risk of developing HCC is 100 times higher in patients with persistent HBV infection compared to non-infected individuals (96; 85). Other risk factors for HCC include cirrhosis, alcoholism and inherited conditions such as hemochromatosis and alpha-1-antitrypsin deficiency (95).

HBV has eight genotypes (A-H), which were classified based on the divergence of ≥ 8% of the entire genome. The HBV genotypes have distinct geographical distribution as summarized in table 1. Several studies suggested that HBV genotypes may influence the clinical outcome of

the HBV infection (85). For example, in Taiwan genotype C has been reported to induce HCC much later than genotype B. However, genotype C is linked to more severe liver disease including cirrhosis and HCC, whereas genotype B is associated with HCC in non-cirrhotic patients. In relation to treatment, it was demonstrated that genotype C has a lower response to interferon therapy compared to genotype B (97). Another study conducted in Europe showed that genotype A responds better to interferon treatment than other HBV genotypes (85).

The pathogenesis of HBV in HCC may be a direct or indirect effect of the virus. The indirect effects include the process of the inflammation, regeneration and fibrosis associated with cirrhosis due to the HBV infection (95). HBV DNA has been shown to become integrated into the DNA of infected hepatocytes. The insertion of HBV DNA into host genome can create an unstable environment, which may induce genomic alterations or may lead to the production of altered protein products; these events can facilitate hepatocyte malignant transformation. In addition, the expression of HBV proteins such as surface proteins and the X protein (HBx) may have direct effect on cellular functions, leading to the tumour formation (98).

Table 1: Geographic distribution of HBV genotypes. Adapted from ref. (85).

Distributions	Genotypes
White Caucasians in Europe, Black Americans in US (Ae), Black Africans in South Africa (Aa), Asia (Aa), India	A (Aa, Ae)
Southern China (Ba), Taiwan (Ba), Vietnam (Ba), Asians in the USA, Japan (Bj)	B (Ba, Bj)
China (Mainland and Taiwan), Japan, Thailand, Asians in the USA	C
White Caucasians (Southern Europe), Arabs (North Africa and the Middle East), India	D
West Africa	E
Central and South America	F
United States, France	G
Central America	H

1.6.3. Clinical course of HBV and HIV co-infection

In general, HBV tends to be more aggressive in HIV infected patients than in HIV uninfected patients. HIV infected patients have higher rate of HCC than HIV uninfected patients. HIV infection alters the course of chronic hepatitis B infection by increasing serum HBV DNA concentrations, declining levels of liver enzymes and faster development of liver cirrhosis,

particularly in patients with low CD4 T cell counts. Low CD4 T cell counts decrease the inflammatory response to chronic hepatitis B (7). The consequences of HIV and HBV co-infection can also include the effects of the HIV antiretroviral treatment in the disease course caused by HBV. Studies from Thailand and Taiwan showed that HIV and HBV co-infection increases the risk of hepatotoxicity from antiretroviral drugs by three to five times. Another issue, the interruptions or changes in HIV ART, can cause emergence of drug-resistant HBV. Furthermore, HIV ART may cause exacerbation of chronic hepatitis during immune restoration (99).

Controversy exists with regard to the impact of HBV on the disease course caused by HIV. Some studies have suggested that HBV can negatively affect the recovery of $CD4^+$ T cells in HIV infected patients (100) while others did not find any impact on $CD4^+$ T cells depletion and progression to AIDS (101).

1.6.4. Viral Structure and replication cycle of HBV

The HBV particle is a spherical enveloped DNA virus with a diameter of 42 nm, which belongs to the Hepadnaviridae family. The viral particle consists of a lipid bilayer envelope containing HBsAg, which surrounds the nucleocapsid core (HBcAg). The nucleocapsid core contains the viral DNA genome and enzymes used in viral replication (DNA polymerase) (figure 7). The genome of HBV is a partially double-stranded circular DNA of about 3.2 kilobase (kb) pairs. It is organized in a compact manner with four overlapping open reading frames (ORFs), namely S, C, P, and X. The *S* ORF encodes the viral surface envelope proteins (HBsAg). The *C* ORF encodes the viral nucleocapsid core (HBcAg) or hepatitis B e antigen (HBeAg), depending on whether translation is initiated from the core or precore regions of the *C* genes. *P* and *X* ORFs encode the DNA polymerase and a 16.5-kd protein (HBxAg) with multiple functions (signal transduction, transcriptional activation, DNA repair, and inhibition), respectively (Figure 8). The DNA polymerase is functionally divided into three domains: the terminal protein domain, the reverse transcriptase (RT) domain and the ribonuclease H domain, which are involved in encapsidation and initiation of minus-strand synthesis, catalyzes genome synthesis, and degrades pregenomic RNA and facilitates replication, respectively (94).

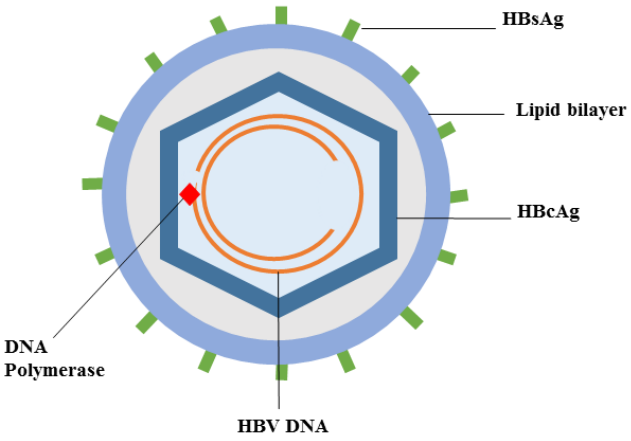

Figure 7: Structure of HBV mature virion particles.

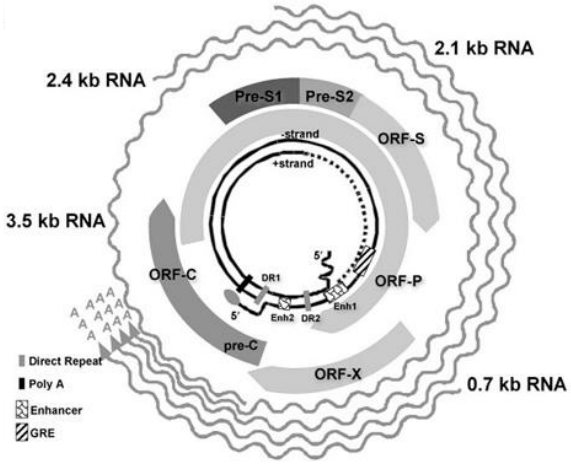

Figure 8: The organization of HBV genome, key regulatory elements and RNAs. Reprinted with permission from (94).

Hepatocytes are the only confirmed site for HBV replication (102). The HBV virions, likely using the pre-S domain of the surface protein, bind to unknown hepatocyte surface receptors. Following the binding of the virus to the host cells, fusion takes place which results in the release of the viral cores into the cytoplasm. The viral cores are transported into the nucleus where the virus DNA turns into a covalently closed circular form (cccDNA). The cccDNA is transcribed by the host RNA polymerase to produce all viral RNAs necessary for protein production and viral replication. The viral RNAs are transported out to the cytoplasm and then are translated into different viral proteins. Core particles are then assembled in the cytosol with a single pregenomic RNA and viral DNA polymerase packed with core proteins. The viral

genome is then synthetized by reverse transcription of the pregenomic RNA. A large proportion of the core's particles are coated with viral lipoprotein envelopes and the virion assembly is exported from the cell as a mature infectious virus, while a small portion of core particles are sent back to the nucleus to maintain a stable pool of the cccDNA (figure 9). The integration of the viral genome into the host genome is not necessary for the HBV replication (98).

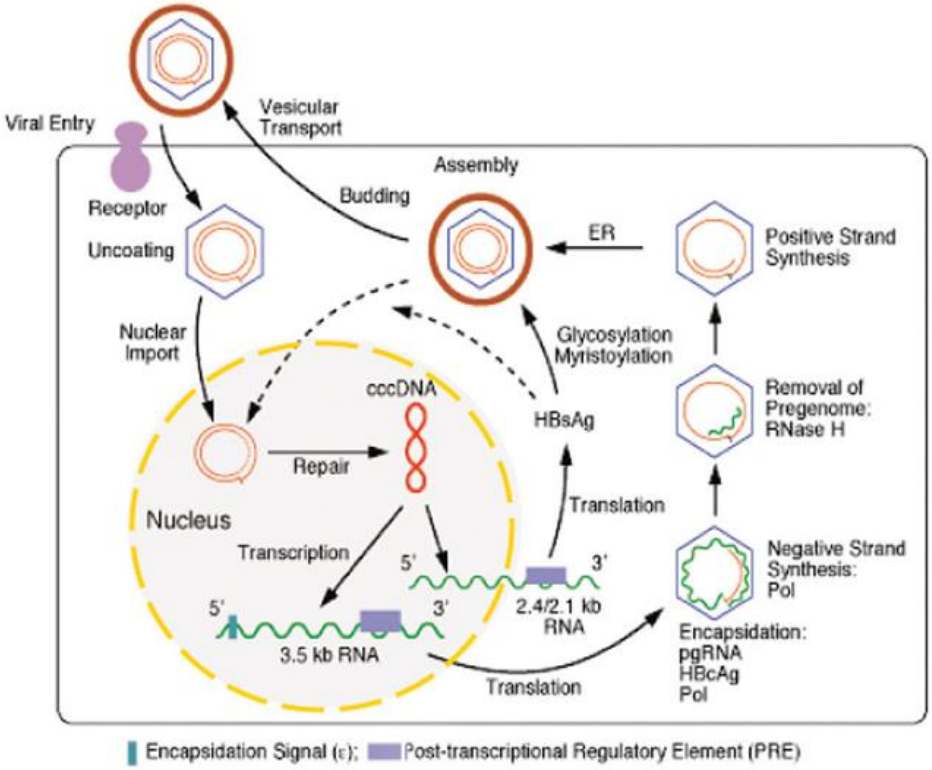

Figure 9. HBV life cycle. Reprinted with permission from (94).

1.6.5. Mode of HBV transmission

Humans are the only natural host of HBV. HBV is spread through contact with infected blood and other body fluids. In high endemicity areas, the most common route of transmission is perinatal during pregnancy or horizontal in early childhood, particularly in China and Southeast Asia, and sub-Saharan Africa, respectively. In intermediate endemicity areas HBV transmission is either perinatal or horizontal (103) and occur in all age groups (85). In low endemicity areas, HBV is acquired by horizontal transmission during adolescence or adulthood (103), in well-defined high-risk groups, which include sex workers and homosexual males, injection drug users, health care workers, and patients submitted to regular blood transfusions (85). The risk

of HBV acquisition through blood transfusion has been greatly reduced due to the improvement of diagnostic tests and progressively broader screening for HBV infection (103; 85).

1.6.6. Diagnosis of HBV infection

The diagnosis of HBV infection is based on serological testing for the detection of antibodies and antigens. Serologic tests are commercially available for all markers except HBcAg because no free HBcAg circulates in blood.

On-going HBV infection is diagnostic by the presence of HBsAg. Acute infection is identified by the presence of HBsAg only, since this is the only serologic marker detected during the first 3–5 weeks after infection. HBeAg can be detected in acute or chronic HBV infection. The presence of HBeAg indicates a high level of virus replication and high degree of infectivity.Life exposure to HBV (present or past HBV infection) is identified by the detection of total Anti-HBc in serum using assays that detect IgM and IgG class antibodies. The presence of the IgM class of anti-HBc in serum is an indication of acute HBV infection. Chronic HBV infection is diagnostic by the detection of HBsAg and total anti-HBc (93).

HBV DNA assays are available and they are useful for quantifying the HBV viral load and monitoring response to treatment (104).

1.6.7. Prevention of HBV infection

The main strategies available for the prevention of HBV infection are: behaviour modification, passive immunoprophylaxis and active immunization (85).

Behaviour modification, which includes changes in sexual practice and screening of blood products, has reduced the risk of HBV infection. However, this strategy has a greater impact in developed countries than in developing countries, where HBV transmission mainly occurs in neonates and children in early childhood. In these age groups, passive and active immunoprophylaxis will be more effective (85). Additionally, HBV screening in pregnant women should be introduced in developing countries to reduce the risk of HBV mother-to-child transmission.

Passive immunoprophylaxis is used in four situations: (1) new-borns of HBV infected mothers, (2) after needlestick exposure, (3) after sexual exposure, and (4) after liver transplantation. Immunoprophylaxis is recommended for all infants born from HBV infected mothers. The current dosing recommendation is 0.13 ml/kg HBIG within 12 hours after birth in combination with active immunization. The combination results a 90% level of protection against HBV perinatal acquisition (85).

Active immunization through the HBV vaccine is recommended by WHO, to be included in national immunization systems in countries with an HBV prevalence ≥ 8% (85). By 2015, 184 countries had routine infant immunization with the HBV vaccine and the global coverage with 3 doses of the vaccine was estimated at 83% (105). The complete vaccination induces protective antibody levels in more than 95% of infants, children and young adults. Protection lasts for at least 20 years (11). There are still challenges in order to achieve the goal of universal childhood immunization against HBV, such as poor immunization delivery infrastructure, low coverage and limited financial resources. Therefore, efforts are still needed to improve the global HBV vaccination coverage (85).

The strategy of prevention of HBV infections in Mozambique is based on the national vaccination program, which administrates the HBV vaccine to infants at 2, 3 and 4 months of age (106). Behavioural change is one of the targets of the Mozambique government to prevent STIs.

2. RATIONALE OF THE STUDY

There is consensus that HIV, HBV and syphilis are global health problems, especially in Sub-Saharan Africa, including Mozambique. These infections are sexually transmitted and co-infections are common. The presence of syphilis has been implicated as a risk factor for the acquisition of HIV infections. Syphilis infection may also accelerate the progression to AIDS. Therefore, preventing syphilis infections would contribute to the reduction of HIV transmission or to improve the lives of HIV-infected patients, slowing the progression to AIDS. On the other hand, HIV infection has an impact on the disease course caused by syphilis and HBV infections. Since HIV is one of the major health problems in Mozambique, it is important to prevent more people from becoming infected with HIV, and also protect the general population, and especially preventing HIV infected patients from acquiring syphilis and HBV infections. However, despite the fact that they are preventable infections, especially HBV infections, since a prophylactic vaccine is available, they remain a serious health issue in the country.

Although HIV prevalence is well documented in Mozambique, there is a lack of information on HIV incidence and risk factors associated with infections of virus among the young population, the age group where most of the transmissions occur. Furthermore, despite the magnitude of HBV prevalence in Africa and the world at large, there is no information available on the magnitude of HBV infection and risk factors related to its spread in unvaccinated young adults in Mozambique. HIV co-infection with HBV or syphilis among the youth is also poorly documented in Mozambique. Therefore, the first two studies of this project were performed to provide data on prevalence and/or incidence of these infections, and risk factors related to their spread, which may be useful for designing prevention strategies and for conducting successful clinical intervention studies.

Clinical laboratory reference values may differ significantly between populations and Mozambique, as in many African countries, is still using values derived from populations living in Europe and North America, due to the absence of locally derived values. Therefore, there is a need for studies that establish local reference values and analyse the impacts of using values derived from other populations in Mozambique for patient management, and in clinical studies. In study III, we established for the first time, normal laboratory values in Mozambique.

Many infectious diseases have been successfully controlled by the use of vaccines. This is likely to also be true for HIV, since the available prevention methods are insufficient in

controlling the spread of this virus. Therefore, there is a need to keep searching for a safe, efficacious and affordable vaccine that can control the spread of HIV. The HIVIS and TaMoVac 01 Tanzania trials, which used a HIV-DNA prime and HIV-MVA boost strategy showed strong and broad immune response. In the HIVIS trials the HIV-DNA vaccine was administrated using the Bioject needle-free device, in a dose of 1000 μg per immunization given as 5 injections of 0.1 ml and separating Env and Gag plasmid pools. The TaMoVac 01 study performed in Tanzania, assessed the possibility of simplifying the regime used in the HIVIS studies to 2 injections, using separated and combined plasmids. However, the maximum dose that could be delivered intradermally with the Bioject in 2 injections was 600 μg, since this device can only contain 0.1 ml while the highest concentration of HIV-DNA available was 3 mg/ml. Therefore, a rigorous comparison of 2 vs. 5 injections could not be made. In study IV, we explored the safety, tolerability and immunogenicity of delivering the HIV-DNA vaccine at a concentration of 3 mg/ml, in a volume of 0.2 ml, using a needle free device, the Zetajet™. We compared priming with HIV DNA at a total dose of 600 μg (2 x 0.1 mL of 3 mg/ml) with a higher dose of 1200 μg (2 x 0.2 ml of 3 mg/ml). Boosting vaccinations were performed using HIV-MVA delivered intramuscularly. This was the first study to assess DNA delivery using Zetajet™, in a volume of 0.2 ml. This study also aimed to build capacity for conducting clinical trials and establish, on site, methods to assess the immunogenicity of vaccine candidates.

3. AIM AND OBJECTIVES

General Aims

The primary aim of this project was to improve the control of sexually transmitted infections in a high prevalence region. The secondary aim was to establish a laboratory framework for conducting HIV vaccine trials, and to monitor and characterize cellular and humoral immune responses induced by a HIV candidate vaccine in Mozambique.

Specific objectives

Paper I: To establish a youth cohort in Maputo, Mozambique, define the incidence of HIV and prevalence of HIV, HBV and syphilis in the group, and assess the suitability of the cohort for possible participation in phase I/II HIV vaccine trials.

Paper II: To determine the prevalence and incidence of HBV, the prevalence of exposure, the proportion of chronic carriers and the rates of HBsAg seroclearance in unvaccinated sexually active young adults in Maputo, Mozambique and assess the risk factors associated with HBV infections in the group.

Paper III: To establish reference values for immunology, hematology and chemistry parameters in individuals between 18-24 years old in Maputo, Mozambique and to assess the potential implication of the study derived values in clinical trials.

Paper IV: To compare the safety, tolerability and immunogenicity of delivering HIV-DNA intradermally at a total dose of 600 µg (2 x 0.1 ml of 3 mg/ml) vs. 1200 µg (2 x 0.2 ml of 3 mg/ml) using a needle-free device, the ZetaJet™ followed by two HIV-MVA boosts delivered intramuscularly in healthy Mozambican young adults.

4. METHODS

Table 2. Summary of the study designs used in this thesis.

Paper	Research question	Design
I	Is a youth cohort in Maputo suitable for a phase I/II HIV vaccine trial? A suitable cohort should have low prevalence and incidence of HIV, and low prevalence of other STIs. The cohort should also have low sexual risk behaviours and good retention rate.	Cohort study: Young adults attended at a youth clinic in Maputo, Mozambique. N = 1380
II	What is the prevalence of HBV markers, incidence of HBV and risk factors for HBV infections in Mozambican unvaccinated sexually active young adults?	Cross-sectional study: Young adults recruited from the cohort mentioned above. N = 1377
III	Are the laboratory reference values derived from Mozambican young adults similar to those from European or American population?	Cross-sectional study: Young adults recruited from the cohort mentioned above. N = 257
IV	Will the high dose (1200 µg) of HIV-DNA vaccine have an effect on safety and immunogenicity?	Randomized double-blinded placebo controlled study: Young adults recruited from the cohort mentioned above. N = 24

4.1. Paper I, II and III: Studies Related to the Maputo youth cohort

4.1.1. Study design and population

In preparation for a phase I HIV vaccine trial (TaMoVac 01) in Mozambique (paper IV), we performed a study to define the prevalence and incidence of HIV and other sexually transmitted infections in Maputo, in a population of young adults (paper I). The study took place at the youth clinic of Maputo Central Hospital, between August 2009 and October 2011. In this study, a total of 1380 youths between 18-24 years old, of both genders (1060 females and 320 males), were

consecutively enrolled. At baseline, socio-demographic data, and at each visit, behavioural characteristics and clinical history were recorded using a structured questionnaire followed by a physical examination. HIV, HBV and syphilis tests were performed before enrolment. Participants with negative or indeterminate HIV test results were enrolled in the longitudinal study for determination of HIV incidence. The participants were asked to visit the study site every 4 months during one year. At all visits, dried blood spots (DBS) were collected from the subjects with negative or indeterminate HIV test results and stored for future testing. Pre- and post-test HIV counselling, STI risk reduction counselling and condoms were provided at all study visits. During the study, STI detection and treatment, or referral to another unit was provided for those who needed. CD4+ T-cells count and HIV-1 viral load were performed in all HIV infected participants on the day of diagnosis.

We also determined the prevalence of HBV markers and incidence of HBV in the cohort, and accessed risk factors for HBV infection in this group. In this sub-study, 1377 participants were screened for HBV seromarkers (paper II). Additionally, we established clinical laboratory reference values for this age group using blood sample from 257 healthy individuals (149 females and 108 males) recruited from the same cohort. Only volunteers that were HIV, HBV and syphilis negative and non-pregnant women were enrolled in this cross sectional study (paper III).

4.1.2. Laboratory methods

HIV testing (Study I and II) and determination of incident cases (Study I)

HIV diagnosis was performed using the Determine HIV-1/2 (Inverness Medical, Bedford, United Kingdom) as a screening test, followed by the UniGold HIV-1/2 (Trinity Biotech, Bray, Ireland) as a confirmatory test. We considered as HIV infected individual who were reactive in both assays. Discordant results were defined as indeterminate. To determine the timing of HIV infection in a subject, a reactive HIV rapid assay was performed on a follow-up visit, where the DBS samples collected from the previous visits were tested using DNA PCR (Roche Amplicor HIV-1 DNA test, version 1.5, Roche Molecular Diagnostics, Branchburg, NJ)(DBS- DNA PCR) (figure 10).

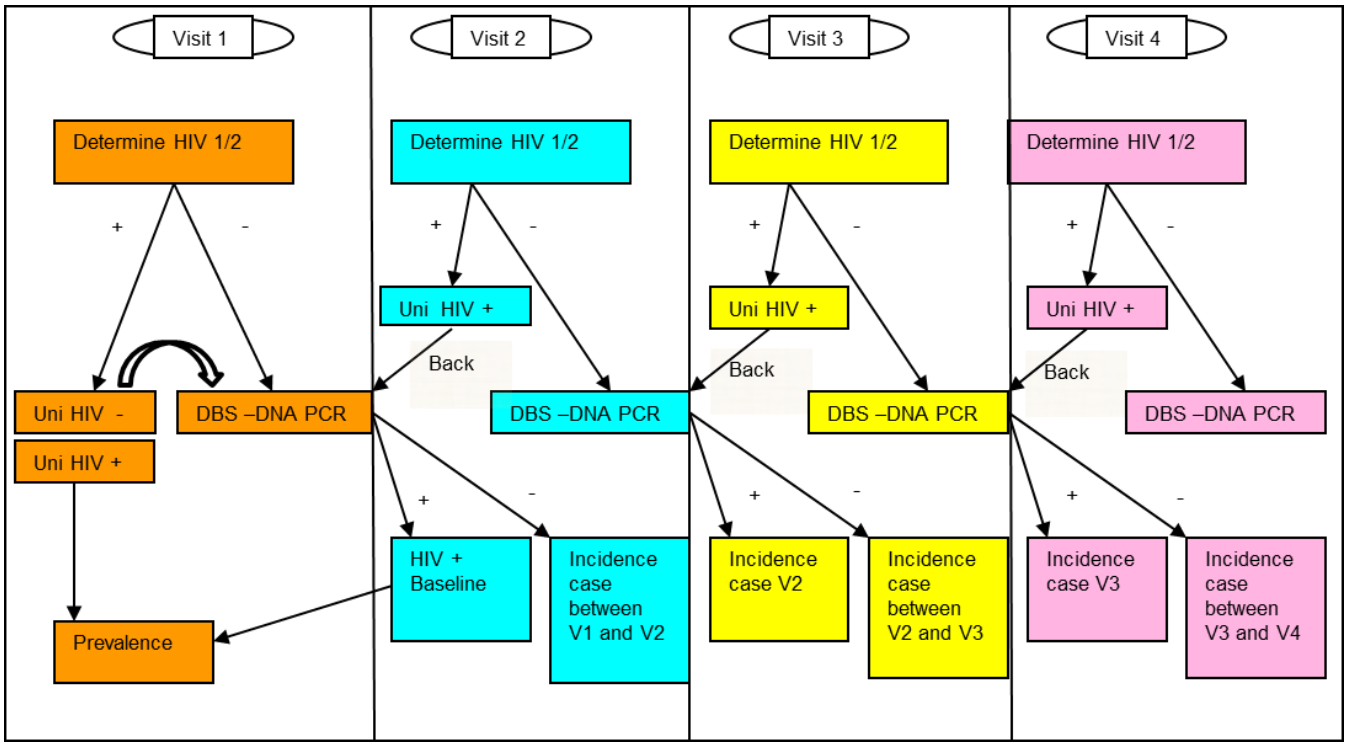

Figure 10: Algorithm for HIV testing and determination of incidence cases.

HIV testing (Study IV)

Two concurrent commercial enzyme-linked immunosorbent assays (ELISA) kits, Murex HIV Ag/Ab (Abbott Murex, Dartford, UK) or GenScreen™ HIV 1/2 version 2 (Bio-Rad, Hercules, California, USA) and Enzygnost anti-HIV-1/2 Plus (Dade Behring, Marburg, Germany) were used for initial HIV screening. For eligibility purposes, both ELISA results were required to be non-reactive. Discordant results on the ELISA assays were resolved using HIV-DNA PCR (Roche Amplicor HIV-1 DNA test, version 1.5, Roche Molecular Diagnostics, Branchburg, NJ). Reactive results were then confirmed by a HIV-RNA PCR assay (COBAS® Taqman®48 analyzer, Roche Molecular Diagnostics, Mannheim, Germany).

HBV testing (Study I and II)

All serum samples were tested for HBsAg and Anti-HBc. Positive samples for Anti-HBc were tested for Anti-HBs and for HBeAg. HBeAg testing was performed using the commercial Elisa HBeAg/Ab Kit (Radim, Pomezia, Italy) and for other markers, Elisa kits from HUMAN GmbH,

Wiesbaden, Germany were used. All tests were performed according to the manufacturers´ recommendations.

Syphilis testing (Study I)

Syphilis diagnosis was performed using the SD Bioline Syphilis 3.0 Rapid Diagnostic Test (Standard Diagnostics, Suwon City, South Korea).

Pregnancy testing (Study I and IV)

The pregnancy testing was done in all females prior to collection of blood samples using the QuickVue One-Step hCG Urine Test (Quidel Corporation, USA).

HIV-1 viral load (Study I and IV)

The HIV-1 viral load was measured using a COBAS Taqman48 analyzer (Roche Molecular Diagnostics, Mannheim, Germany).

Immunophenotyping (Study I, III and IV)

Lymphocyte subset immunophenotyping was performed using a FACSCalibur flow cytometer (Becton-Dickinson, Franklin Lakes, New Jersey, USA). Briefly, 20 µl of CD3 FITC /CD16+CD56 PE/CD45PerCP /CD19APC or CD3FITC/CD8PE/CD45perCP/CD4APC MultiTest reagents (Becton-Dickinson) were added into TruCOUNT tubes (Becton-Dickinson) and then mixed with 50µl of whole blood. The tubes were incubated in the dark, at room temperature for 15 minutes. Red blood cells were then lysed by adding 450 µl of fluorescence-activated cell sorter lysing solution (Becton Dickinson) and incubating for 15 minutes, at room temperature. The samples were then analysed in the Flow cytometer using MultiSET software (Becton-Dickinson). A CD4 count for HIV infected individuals followed the procedure described above *(Study I and IV).*

Complete blood count (CBC) (Study III and IV)

A complete blood count and differential was done using the Sysmex KX-21N Hematology Analyzer (Sysmex Corporation; Kobe Japan) as recommended by the manufacturer. The EDTA blood samples were analyzed within 6 hours of specimen collection. The machine automatically dilutes a whole-blood sample, lyses, counts and gives a printout of the results.

Biochemistry analysis (Study III and IV)

Biochemistry analyses were performed using a Vitalab Selectra Junior (Vital Scientific) according to the manufacturer´s instructions. Serum were separated within 4 hours of collection and analysed within 7 hours of the blood draw. Each sample was analysed for creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), bilirubin, albumin, glucose, uric acid, urea, amylase, HDL cholesterol, triglycerides and alkaline phosphatase (ALP).

4.1.3. Statistical analysis

The statistical methods used are described in detail in each paper. In papers I and II, statistical analyses were performed using Stata, version 12 and 14, respectively. The participants were characterized according to target variables using descriptive statistical methods. The prevalence of HIV, syphilis and HBV (HBsAg) infection, and of other HBV seromarkers was defined as the percentage of positive results and a 95% confidence interval (CI) was calculated for each prevalence rate.

The HIV incidence rate (IR) was calculated as the number of new HIV cases divided by the person-years (PY) of observation accumulated by the cohort. The age-specific changes of prevalence of the serologic markers HBsAg and Anti-HBc was used to estimate the incidence of HBV by using catalytic modelling.

Associations between HIV and HBV seromarkers (HBsAg and Anti-HBc) and possible risk factors (socio-demographic, sexual and behavioural) were tested with the chi-square test. The magnitude of associations was assessed using odds ratios (OR) for HIV and prevalence ratios (PR) for HBV markers with respective 95% CI, and logistic regression models were used to control target variables. A two-sided $P < 0.05$ result was considered statistically significant.

In paper III, data analysis was performed using R statistic software (version 3.0.0). Median and 95% reference ranges (2.5th- 97.5th percentiles) were established for all parameters analysed. The Mann-Whitney U test was used to determine differences by gender. For all analysis the significance was set at 0.05. For the parameters that did not meet the recommended minimum sample size of 120 individuals recommended by the Clinical and Laboratory Standards Institute (CLSI) (107), percentiles were obtained from a bootstrap procedure as described previously (108; 109).

4.1.4. Ethical considerations

Approvals were granted by the National Health Bioethics Committee of Mozambique Ref. 380/CNBS/08 and amendments: Ref. 422/CNBS/08, Ref. 148/CNBS/09, Ref. 84/CNBS/10, Ref. 143/CNBS/11 and Ref 117/CNBS/12. Written informed consent was obtained from each participant prior to conducting any study procedures.

4.2. Paper IV: Phase I HIV vaccine trial in Mozambique

4.2.1. Study design and population

The safety and immunogenicity of intradermal HIV-DNA priming at a dose of 600µg or 1200µg followed by intramuscular HIV-MVA boosts was evaluated in a phase I HIV vaccine trial conducted in Maputo, Mozambique – TaMoVac 01. The trial took place at the Polana Caniço Health Research and Training Centre in Maputo, from August 2011 to March 2013. Twenty-four healthy volunteers, aged 18-26 years, were recruited at the Youth Clinic of the Maputo Central Hospital. The recruited volunteers participated in the cohort study reported in paper I. The participants were randomized into two groups either to receive the low or high dose of HIV-DNA vaccine using a needle-free device, the Zetajet™, followed by two doses of recombinant MVA expressing HIV genes using syringe. Within each group, subjects were randomized to receive the vaccine or placebo (normal saline) at a ratio of 5:1. The study team and participants were blinded to the randomization groups (Figure 11 and table 3).

4.2.2. Vaccines

The HIV-DNA vaccine candidate that was produced by Vecura (Huddinge, Stockholm) is composed of seven DNA plasmids containing different HIV genes: Pool 1 encoding Env subtypes A, B and C and Rev subtype B; and Pool 2 encoding Gag subtypes A and B and RTmut subtype B (110). The HIV-MVA vaccine candidate is a live recombinant non-replicating poxvirus vector that has been genetically engineered to express HIV-1 gp160 (Subtype E, isolate CM235) and Gag and Pol (integrase-deleted and reverse transcriptase non-functional,

Subtype A, isolate CM240) (111). The HIV-MVA vaccine was manufactured at WRAIR, Rockville, USA.

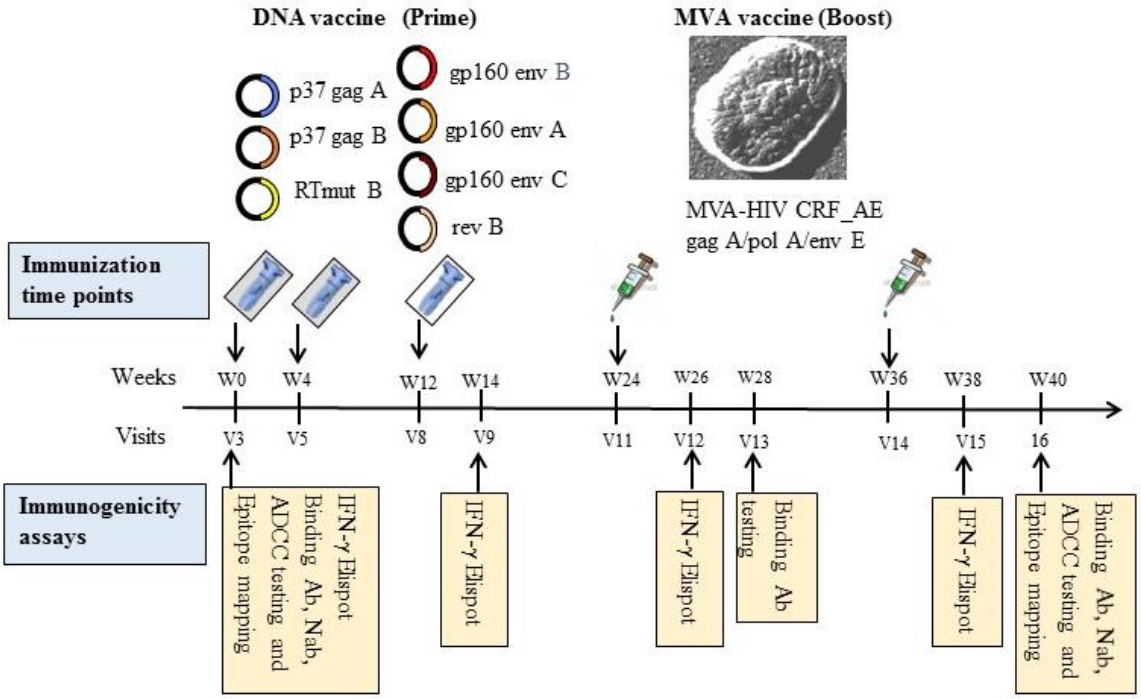

Figure 11: Vaccines, immunization time points and immunogenicity assays.

Table 3: Randomization, dose and study groups.

Group	Number of Volunteers	DNA immunization Weeks 0, 4 and 12	MVA boost Weeks 24 and 36
IA	10	600 µg i.d. (2 x 0.1 ml)	MVA, at 10^8 pfu i.m.
IB	2	Saline i.d. (2 x 0.1 ml)	Saline, i.m.
IIA	10	1200 µg i.d. (2 x 0.2 ml)	MVA, at 10^8 pfu i.m.
IIB	2	Saline i.d. (2 x 0.2 ml)	Saline, i.m.

4.2.3. Safety evaluations

Safety assessment was performed clinically and by laboratory safety tests. Thirty minutes after vaccination, the participants were observed for vital signs and local and systemic reactogenicity. Subjects were asked to make a daily record of any reaction in the post-vaccination diary card for seven days after each immunization, and to contact the clinic if any moderate or severe reaction occurred. ECG was performed at screening and 2 weeks after each HIV-MVA immunization as per the US FDA requirements. Blood samples for routine laboratory tests were collected 2 weeks after each immunization. The laboratory safety tests included CBC, ALT, creatinine, random blood glucose, and total and direct bilirubin. Pregnancy test for females, urinalysis and HIV tests for all were performed at screening, prior to each vaccination and at the last visit. The participants were required to have normal urinalysis results prior to each vaccination. Participants with positive HIV and pregnancy test results after enrolment were considered ineligible for vaccination, but were followed until the end of the trial, or post-delivery for pregnant women, for safety assessments.

All adverse events (AEs) that occurred during the trial were recorded. The DAIDS toxicity tables (version 1.0, December 2004, clarification August 2009) were used for grading the clinical and laboratory AEs.

4.2.4. Immunogenicity evaluations

Cell mediated immune (CMI) responses was assessed by the IFN-γ ELIspot assay, using fresh PBMCs collected at baseline, two weeks after third HIV-DNA immunization, and two weeks after the first and second HIV-MVA boost. HIV-1-specific peptide pools representing the DNA vaccine subtype A and B Gag, and MVA vaccine CRF01_AE Gag, Env and Pol were used. Serum samples were used in all antibody assays. Binding antibodies assay was performed using samples collected at baseline, four weeks after the first and second HIV-MVA boost, and HIV proteins (recombinant HIV-1CN54 clade C gp140 (Centre for AIDS Reagents, NIBSC Potter Bar, UK) and native gp160 subtype B (HIV-1IIIB, Advanced Biotechnologies Inc., Columbia, MD) in in-house ELISAs. NAb, Antibody-dependent Cellular Cytotoxicity (ADCC) and epitope mapping assays were performed using samples from baseline and four weeks after the second HIV-MVA boost immunization. NAb were measured using TZM-bl assay with pseudoviruses SF162.LS (subtype B) and 93MW965.23 (subtype C) and using PBMC assay with infectious molecular clone (IMC) SF162.LS (subtype B) and CM244 (CRF01_AE). ADCC activity was measured

using assay employing Env.IMC.LucR virus-infected cells (CM235 CRF01_AE IMC) as targets. Figure 11 shows the vaccines, immunization time-point and immunogenicity endpoints.

Peptide microarray

HIV-1 Env-specific IgG was assessed using microarray slides from JPT Peptide Technologies GmbH (Berlin, Germany). Each array consisted of 8 full-length globally representative Env sequences, CN54gp140 and MVA-CMDR encoded Env immunogens, and additional peptide variants for hot spots of IgG recognition. The microarray slides were placed in incubation chambers. Arrays were blocked with 990 µl of Superblock T20 PBS blocking buffer (Thermo Scientific) for 10 min at room temperature (RT) on a shaker. Then, 10 µl of each plasma sample was added to Superblock, diluting the sample 1:100. Arrays were then incubated for 2h at RT on a shaker. After incubation, arrays were washed five times with PBS containing 20% Tween, for 3 min on a shaker for each wash. Arrays were then incubated for 1h at RT on a shaker with a secondary antibody, 1 mg/ml anti-IgG Cy5 antibody, diluted 1: 5000 with Superblock T20. Arrays were then washed 5 times with PBS and then 5 times with de-ionized water, for 3 min on a shaker for each wash. The arrays were let to dry at RT in the dark and scanned with a Genepix 4000 Scanner (Axon Instruments, Union City, Calif.) at a wavelength of 650 nm and 532 nm lasers, at 500 PMT and 100 laser power.

Microarray data analysis

The raw images were analyzed using Genepix Pro 6.0 software (Molecular Devices) and Array List (GaL) file, provided by JPT creating a Genepix Results (GPR) file that contained information about the fluorescent intensity of each feature, with the name and position on the slide. R scripts software was then used to calculate the Mean fluorescent intensity (MFI) of the triplicates of each feature (peptide spot). The fluorescent intensities measured post-vaccination were corrected by subtracting the baseline value for that sample. The corrected fluorescent intensity was then compared to the threshold for positivity (2500 MFI), and all values above the threshold were considered positive.

Results from the R analysis were transferred to an excel file and the frequency of responders were calculated for each positive peptide intensity in the vaccinees and in the study groups. To assess the magnitude of responses, the MFI for each positive peptide in the study groups was determined. Subsequently, analysis of significance was performed to identify antigen features (sequences) with statistically significant reactivity in each study group related to paired samples (pre- and

post-vaccination samples). These calculations were performed with R scripts software and P-value < 0.05 was considered statistically significant. Sequences with statistically significant antibody reactivity were introduced in the HIV-1 sequence database to identify the amino acid start position aligned to the HXB2 HIV-1 strain.

4.2.5. Study endpoints

The study safety endpoints were any grade 3 or above, clinical or laboratory (only if clinical significant), adverse event that occurred after the first immunization until the last study visit. The primary immunogenicity endpoint of the trial was IFN-γ ELISpot responses to either Gag or Env peptide pools, two weeks after the first and second HIV-MVA vaccinations. The secondary immunogenicity endpoints were the magnitudes of the IFN-γ ELISpot responses to Gag or Env peptide pool stimulation, determined two weeks after the first and second HIV-MVA vaccination, and the antibody responses to HIV-1 subtype C gp140 and subtype B gp160, NAb, ADCC activity and IgG binding antibodies against HIV-1 Env regions were determined four weeks after the second HIV-MVA vaccination.

4.2.6. Statistical analysis

Clinical and safety laboratory data were double entered in a MySQL database and immunological data in Excel under study code. Data was exported and analyzed in SPSS 21.0. Descriptive statistics were used to summarize baseline characteristics. Categorical variables were expressed in percentages and continuous data as means with respective standard deviations (SD).

Most immunological data was presented without statistical analysis, as this was a descriptive, hypothesis-generating study. The median magnitude of IFN-γ ELISpot responses and antibody titers were compared using the Mann-Whitney U-test. Fischer's Exact test was used for comparison of frequencies of responses between groups. A p-value of < 0.05 was considered statistically significant.

4.2.7. Ethical considerations

The trial was approved by the National Health Bioethics Committee of Mozambique (ref. 76/CNBS/11 and 142/CNBS/11) and by the Regional Ethics Committee, Stockholm, Sweden (2011/1684-31-4). Written informed consent was obtained from each study participant. The participants were required to have passed a test of understanding before screening procedures were initiated.

5. MAIN FINDINGS

5.1. Paper I

5.1.1. Suitability of the youth cohort in Maputo for phase I/II HIV vaccine trials

A total of 1380 youths, 320 (23.2%) males and 1060 (76.8%) females, were enrolled in the study. The age of the participants ranged from 18 to 24 years (mean was 20.9, SD ± 1.71), with males being older than females (21.7 vs. 20.7, $p < 0.001$). The overall prevalence of HIV-1 at baseline was 5.1% (95%CI: 3.97–6.31). Females had a significantly higher prevalence of HIV-1 (5.8%) as compared to males (3.1%), $p = 0.018$. The overall incidence of HIV-1 was 1.14/100PY (95% CI: 0.67–1.92). In our study, all incident cases occurred in females. The HIV incidence rate among women was 1.49/100 Women Years (WY) (95%CI: 0.88–2.51). Retention rates were stable throughout the study, being 85.1% at the last visit. Figure 12 shows the HIV infections among participants and retention during the study.

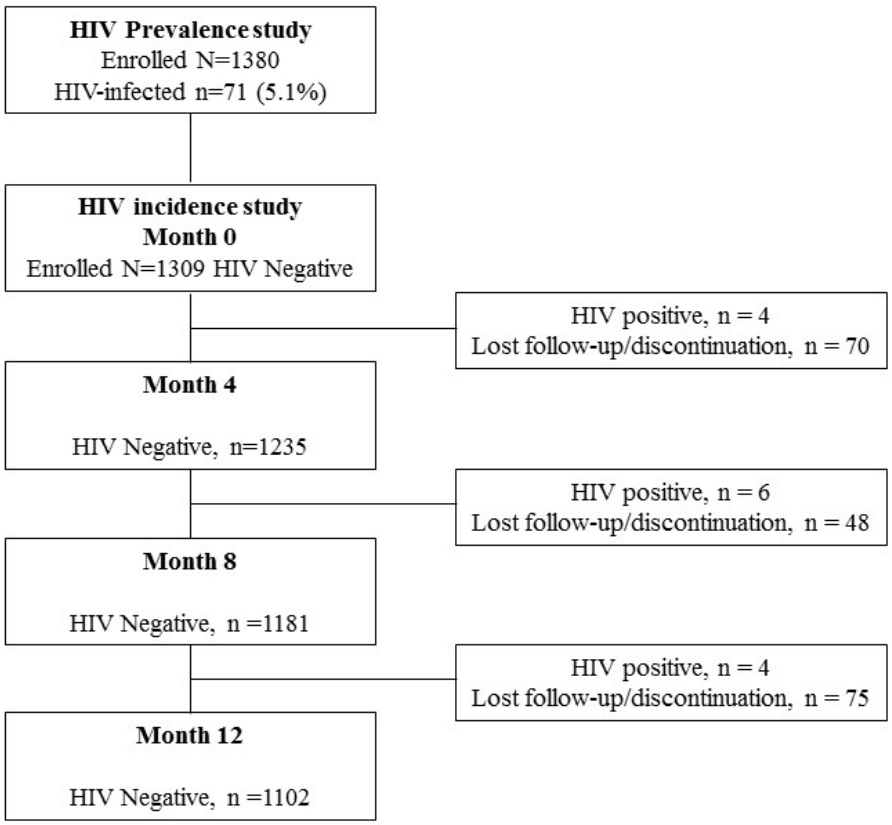

Figure 12: HIV infections among participants and retention during the study.

5.1.2. HBV and syphilis prevalence, and co-infections with HIV in young adults in Maputo, Mozambique

Overall HBsAg prevalence in this study was 12.2% (95%CI: 10.5%–14.0%) with males having a higher prevalence of HBV infection than females (15.9% vs. 11.1%, p = 0.02). The overall syphilis prevalence was 0.36% (95%CI: 0.15%–0.84%). Here, 11.3% of HIV-infected participants were also infected by HBV and all participants with a reactive syphilis test had other sexually transmitted viral infections (HIV and/or HBV).

5.2. Paper II

5.2.1. Prevalence of HBV seromarkers in young adults in Maputo City, Mozambique

In paper II a more detailed analysis of markers of HBV infection was performed. A total of 1377 volunteers were screened for HBV seromarkers, among them 1057 (76.8%) were females and 320 (23.2%) were males. The prevalence of HBV exposure (Anti-HBc), HBV infection (HBsAg), chronic carriers (HBsAg and Anti-HBc), and acute infection (HBsAg only) were 42.8%, 12.1%, 8.5% and 3.6%, respectively. The prevalence of HBsAg and of Anti-HBs was significantly higher among men than in women, 15.9% vs. 11.0%, p=0.016 and 48.8% vs. 41.0%, p=0.012, respectively. There was no statistical difference between genders with regard to the prevalence of chronic HBV infection.

All volunteers with anti-HBc positive results were tested for anti-HBs and HBeAg. The prevalence of these markers was 45.5% and 6.8%, respectively. The prevalence of anti-HBs was higher in females than in males (46.7% vs. 42.5%) and the frequency of HBeAg was higher in males than in females (7.8% vs. 6.5%), although, these differences did not reach statistical significance (table 4). Figure 13 shows the algorithm for HBV seromarkers detection.

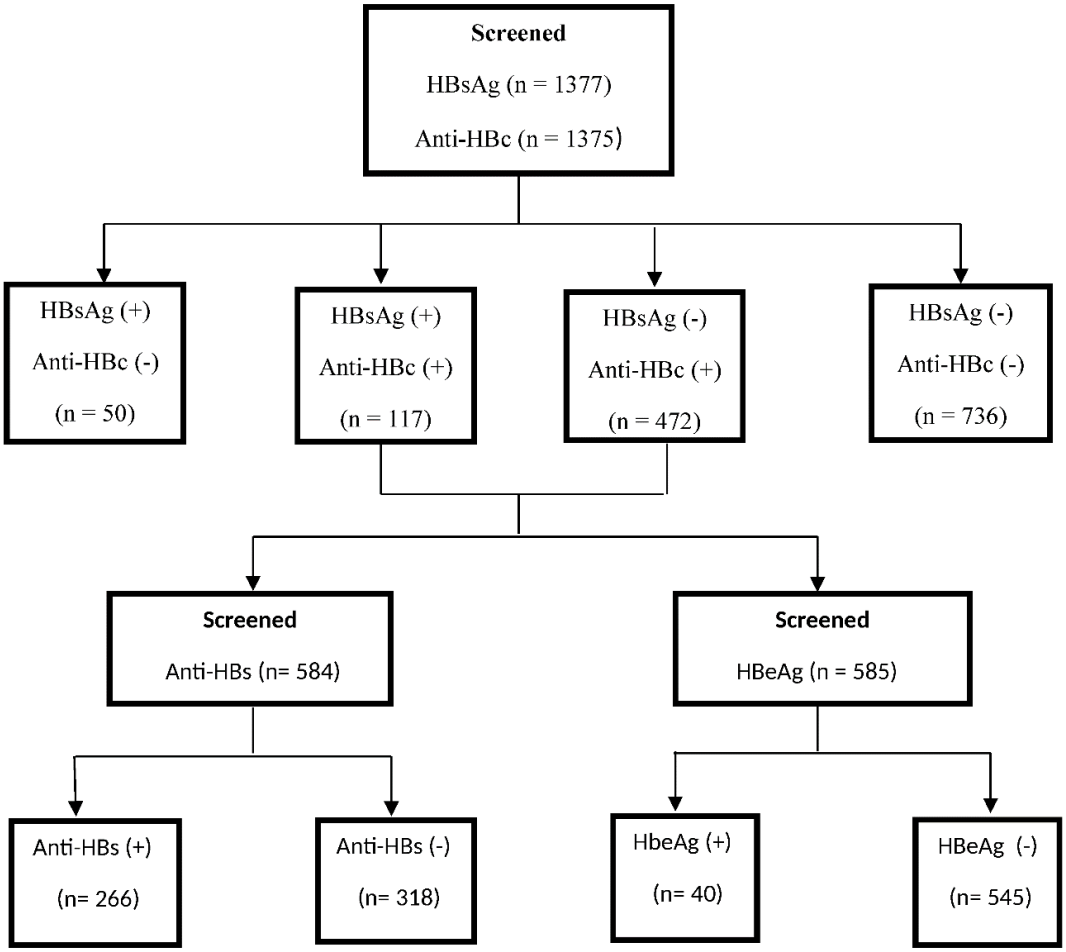

Figure 13: Algorithm for HBV seromarkers detection.

T**able 4:** Prevalence of HBV seromarkers among young adults in Maputo City, Mozambique.

HBV seromarkers	Female		Male		Total		p-value
	Tested	Prevalence (95% CI)	Tested	Prevalence (95% CI)	Tested	Prevalence (95% CI)	
HBsAg	1057	11.0 (9.2 - 13.0)	320	15.9 (12.1 - 20.4)	1377	12.1 (10.4 - 14.0)	**0.016**
Only HBsAg	1057	3.2 (2.2 - 4.5)	320	5.0 (2.9 - 8.0)	1377	3.6 (2.7 - 4.8)	0.137
Anti-HBc	1055	41.0 (38.1 - 44.1)	320	48.8 (43.2 - 54.4)	1375	42.8 (40.2 - 45.5)	**0.012**
HBsAg and Anti-HBc	1055	7.8 (6.2 - 9.6)	320	10.9 (7.7 - 14.9)	1375	8.5 (7.1 - 10.1)	0.075
HBeAg	431	6.5 (4.4 - 9.3)	154	7.8 (4.1 - 13.2)	585	6.8 (4.9 - 9.2)	0.584
Anti-HBs	431	46.7 (41.8 - 51.5)	153	42.5 (34.5 - 50.7)	584	45.5 (41.5 - 49.7)	0.385
HBsAg(+), AntiHBc(+) and HBeAg(+)	431	5.3 (3.4 - 7.9)	154	5.8 (2.7 - 10.8)	585	5.5 (3.4 - 7.6)	0.812
HBsAg(-), Anti-HBc(+) and Anti-HBs (+)	431	36.9 (32.3 - 41.6)	153	32.0 (24.7 - 40.0)	584	35.6 (31.7 - 39.7)	0.290

5.2.2. Incidence of HBV in young adults in Maputo City, Mozambique

The incidence of HBV was estimated using the age changes of prevalence of the serologic markers HBsAg and Anti-HBc using catalytic modelling. The overall HBV incidence rate was 180 (95% CI: 130 – 230) per 100,000 PY when we used the prevalence of HBsAg in the model. We did not find statistically significant differences between genders in the incidence rates estimated using the prevalence of HBsAg, p = 0.168. The HBV incidence rate was estimated to be 2690 (95% CI: 2470-2920) per 100,000 PY when we used the prevalence of Anti-HBc in the model. In this case, males had a significantly higher HBV incidence rate compared to females, 3130 (95% CI: 2670 - 3670) per 100,000 PY vs. 2560 (95% CI: 2320 - 2810) per 100,000 PY, respectively, p = 0.033.

5.3. Paper III

5.3.1. Clinical laboratory reference values for Mozambican young adults and comparison with those from the same age group in other African countries and values from the US

A total of 257 youths (102 males and 155 non-pregnant females), HIV, HBV and syphilis negative were enrolled in the study. Due to low attendance of males at the Youth Clinic, the target of > 120 males was not reached. The full range of lymphocyte subsets, hematology and chemistry analytes was not run in all subjects, primarily due to insufficient access to reagents required to test some parameters. However, a robust bootstrap analysis was used to eliminate bias due to the small sample size.

Analyses of immunological parameters revealed statistically significant differences between genders in some parameters. Most relevant, females had significantly higher values of absolute (824 vs. 713 cells/µl, p<0.0001) and percentage (40.4 vs. 35.5 cells/µl, p<0.0001) CD4+ cells count, and CD4:CD8 ratio (1.7 vs. 1.5, p=0.0001) than males. The immunology reference ranges derived from this study were comparable to those reported for the USA and western Kenya. The hematological values derived from this study differed from the USA values. In most cases, the study-derived values were lower than those derived from the USA population. More than 50% of the participants had Hb values that were outside the lower limits of the USA reference range, among them 69.3% were female. Our values were similar to reports of populations in western Kenya and Uganda. The chemistry ranges derived here were lower

compared to those from western Kenya in the same age group. The Maputo values were comparable to USA values, with a few exceptions. The upper limits of the study reference ranges for ALT, AST, bilirubin and cholesterol were somewhat higher than those from the USA. Table 5 shows the comparison of laboratory reference ranges (of the most important parameters used in clinical studies to assess the safety of the study products) from Maputo, western Kenya, Uganda and USA.

Table 5: Comparison of laboratory reference ranges derived from young adults in Maputo, Mozambique compared with those from western Kenya and the United States of America.

Parameter	Maputo-Moz. (18-24 years old)	Western Kenya (18-34 years old)	Uganda (19-24 years old)	USA
Hemoglobin (g/dL)				
Male	12.3 – 16.0	11.4 – 16.9	11.5–17.1	13.5-17.5
Female	7.3 – 13.2	8.0 – 14.2	9.9–13.7	12.0-16.0
Platelets (10^6 cells/µl)	125.2 – 488.0	[a] 103 - 390	NA	150 - 350
WBC (10^6 cells/µl)	3.0 – 8.7	[a] 3.3 – 9.3	3.7–9.7	4.5 – 11.0
Neutrophils (10^3 cells/µl)	1.2 – 6.1	[a] 0.9 – 5.2	1.0–3.5	1.8 – 7.7
Lymphocytes (10^3 cells/µl)	1.1 – 3.1	[a] 1.1 – 3.5	1.3–4.1	1.0 – 4.8
T CD4 (Cells/ µl)	381 – 1340	444 – 1488	NA	404 - 1612
ALT (U/L)	5.0 – 48.2	7.2 – 61.3	NA	0 - 35
AST (U/L)	13.7 – 42.8	13.8 – 50.4	NA	0 - 35
T-Bil (µmol/L)	4.4 – 27.9	5.1 – 40.7	NA	5.1 – 17.0
Creatinine(µmol/L)	47.1 – 103.2	50 – 113	NA	0 - 133
Glucose (mmol/L)	3.1 – 5.5	2.1 – 6.6	NA	4.2 – 6.4

[a]Values corresponding to individuals aged 13–34 years.

NA – Not available.

5.3.2. Implications of the study derived reference values in clinical trials

A comparison between the study derived values and those from the USA showed higher variations in most values, especially in hematological parameters. Overall, 159 (62%) of the study participants would have been excluded in potential clinical studies due to abnormal laboratory values, if USA reference ranges were used in this group as inclusion and/or exclusion criteria to assess participant eligibility. However, if the local reference ranges were used, only 40 (16%) of the participants would have been excluded. The hematological and clinical chemistry parameters would have been the most important cause of participant exclusion. The DAIDS toxicity tables for grading adverse events, which have been used in many clinical trials,

predicted 297 adverse events in the laboratory values obtained from the blood samples of the study participants. In respect to the hematologic parameters, Hb and neutrophil counts accounted for the majority of the AEs, 32 and 16 AEs, respectively. Among the clinical chemistry parameters, ALT and AST would have resulted in 27 and 197 AEs, respectively (table 6).

Table 6: Frequency of predicted adverse events in the youth cohort based on a comparison with DAIDS values.

Parameter		Ineligible per US Comparison Interval	DAIDS toxicity grading			
			Grade 1	Grade 2	Grade 3	Grade 4
	N	n (%)	n (%)	n (%)	n (%)	n (%)
Hemoglobin (g/dL)						
Male	100	14 (14)	1(0.9)	0 (0)	0 (0)	0 (0)
Female	150	104 (69.3)	20 (13.2)	7 (4.6)	4 (2.6)	0 (0)
Platelets (10^6 cells/µl)	253	47 (18.6)	3 (1.2)	4 (1.6)	0 (0)	0 (0)
WBC (10^6 cells/µl)	252	64 (25.4)	1 (0.4)	0 (0)	0 (0)	0 (0)
Neutrophils (10^3 cells/µl)	254	24 (9.4)	14 (5.5)	2 (0.8)	0 (0)	0 (0)
Lymphocytes (10^3 cells/µl)	247	3 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
T CD4 (Cells/ µl)	226	4 (1.8)	6 (2.7)	1 (0.4)	0 (0)	0 (0)
ALT (U/L)	253	15 (5.9)	22 (8.7)	5 (1.9)	0 (0)	0 (0)
AST (U/L)	253	14 (5.5)	145 (57.3)	52 (20.6)	0 (0)	0 (0)
T-Bil (µmol/L)	253	38 (15.0)	5 (1.9)	1 (0.4)	0 (0)	0 (0)
Creatinine(µmol/L)	253	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Glucose (mmol/L)	251	104 (41.1)	0 (0)	0 (0)	0 (0)	0 (0)

5.4. Optimizing the dose of the HIV-DNA prime vaccine - Paper IV

5.4.1. Comparison of vaccines safety among participants who received low doses (600µg) and high doses (1200µg) of the HIV-DNA vaccine

Of the 24 enrolled subjects, 23 (96%) and 21 (88%) reported at least one local and one systemic solicited adverse event (AE) during the trial, respectively. Most (94.8%) of these events were mild. The common local solicited AE in vaccines was pain, which occurred in 18 of 20 (90.0%) participants. The most common systemic solicited AE was a headache, occurring in 17 of 20 (85.0%) participants. There were 166 unsolicited clinical AEs, 165 non-serious and one serious AE, reported by vaccine recipients during the course of the trial. The serious AE, HIV infection, was reported in a male subject in the high HIV-DNA dose group. The HIV test result at the time of the first HIV-MVA boost was indeterminate and confirmation of infection was done 15 days after the vaccination.

There were 88 laboratory AEs reported in the vaccine recipients. All laboratory AEs were considered "not related" or "possibly not related" to the investigational products. No ECG abnormalities were seen after the two HIV-MVA boosts.

Overall, the distribution of AEs was balanced between the vaccination groups.

5.4.2. Comparison of vaccines immunogenicity among participants who received low doses (600µg) and high doses (1200µg) of the HIV-DNA vaccine

HIV-specific cell-mediated immune response

The overall IFN-γ ELIspot responses rate to Gag and/or Env was 14/15 (93%) and 8/10 (80%) two weeks after the first and after the second HIV-MVA boost, respectively. There was no significant difference in response rates to Gag and Env between the low and high DNA dose groups after the HIV-MVA immunizations.

After the HIV-MVA boosts, no difference in median magnitudes of the responses to Gag CMDR was seen between the low and high HIV-DNA immunization groups. IFN-γ responses to Env was significantly higher after the first MVA-HIV boost in the high dose group compared to the low dose group, when comparing all vaccinees (157.5 vs. 420 SFC/million PBMC, p=0.014), and a trend towards a difference was observed when only responders were compared

(150 vs. 420 SFC/million PBMC, p=0.0513). After the second HIV-MVA vaccination no differences in IFN-γ ELIspot responses between study groups were observed. In pair wise analysis of the IFN-γ ELIspot data, Gag CMDR responses were significantly higher after the first HIV-MVA vaccination, rather than after the second HIV-MVA vaccination (median, 360 vs. 142 SFC/million PBMC to Gag CMDR, p=0.0391). The IFN-γ responses to Env did not differ significantly between the two time points (median, 148 vs. 118 SFC/million PBMC), p = 0.6523.

HIV-specific Humoral Immune Responses

At baseline, binding antibodies were not detected among vaccinees. Four weeks after the second HIV-MVA boost, the overall median antibody titer to subtype C gp140 was 800 (range 400-3200) and 400 to subtype B gp160 (range 200-800). The median antibody to subtype C gp140 and to subtype B gp160 was higher in the high dose group than in the low dose group, but the differences did not reach statistical significance, median 2000 vs. 800, p = 0.1993 and median 400 vs. 300, p = 0.1602, respectively.

There was no demonstrable NAb activity in the TZM-bl and PBMC neutralization assays in any of the vaccinees. ADCC-mediating antibodies against to CRF01_AE CM235 were only detected in two (13%) of 16 vaccinees, one in each of the HIV-DNA immunization groups.

5.4.3. Antibody epitope mapping of HIV-vaccine specific responses (preliminary results)

Significance analysis of microarrays

Table 1 shows the list of antigen features (sequences) that had statistically significant antibody reactivity in the vaccinees four weeks after the last HIV-MVA vaccination. Across all vaccinees, Env-specific IgG responses were confined to few antigenic sites in V2, V3 and gp41 immunodominant regions of gp120 protein. A higher number of antigenic sites were recognized by high dose DNA recipients than low dose DNA recipients.

Frequency and Magnitude of IgG binding in peptide microarray

Figure 14 shows the frequency of IgG antibody responses among the vaccinees. Among the vaccinees, 8/16 (50%) had antibodies against at least one antigenic site in the V2 loop. The frequency of responders with responses against the V2 loop was significantly higher in the high dose group than in the low dose group, 6/8 (75%) vs. 2/8 (25%), respectively, p = 0.0486. Visual

inspection of the data identified peptide 176 (HXB2 numbering sequence 164→178) as most frequently recognized in the V2 region among the vaccinees. Of the 16 vaccinees, 9 (56.3%) had antibody responses to the V3 loop and the sequence most frequently recognized was TSIPIGPGQAFYRTG (peptide 326 on array, HXB2 numbering sequence Env 307→321). The IgG response rate to the V3 loop was higher in the high dose group than in the low dose group but without a statistically significant difference (75% vs. 50%, p = 0.1492) (Fig. 2). Antibodies to the gp41immunodominant region were detected in 9/16 (56.3%) vaccinees and the sequence VLAVERYLKDQKFLG (peptide 612 on array, HXB2 numbering sequence Env 580→594) was recognized by all responders. There was no statistically significant difference in the proportion of responders to the gp41immunodominant region between the vaccination groups. Four vaccinees, two in each vaccination group, had antibody responses against the amino acid sequence PDRLGRIEEEGGEQD (peptide 759 on array, HXB2 numbering sequence Env 727→741). Among peptides that had statistically significant antibody reactivity across the vaccinees (175, 325 and 612, peptide positions on array, table 1), we did not find significant differences between groups.

The magnitude of the Env-specific antibody responses was measured by MFI. There was no statistically significant difference between the vaccination groups in the magnitude of responses to any of the Env regions (figure 15).

Table 1. Peptides with statistical significance reactivity in the vaccination groups.

Regions	Position on array	Antigen feature (HXB2)	Sequences	Low-dose group	High-dose group
V2	175	Env 163→177	TEIKDKKQKVHALFY		X
			TELRDKKQRVHALFY		X
			TEIRDKKQRVHALFY		X
			TELRDKKKKVHSLFY		X
			TEIKDKKKKVHALFY		X
			TELRDKKKKVHALFY		X
			TELRDKKRKVHALFY		X
			TELRDKKQKVHSLFY		X
			TELKDKKHKVHALFY		X
V3	176	Env 164→178	ELRDKKQKVHALFYK		X
	322	Env 301→317	NNTRTSIPIGPGQAF		X
	325	Env 304→320	RKSIPIGPGRAFYTT	X	X
			RKSIPIGPGRAFYAT		X
	326	Env 307→321	TSIPIGPGQAFYRTG		X
gp 41 Immunodominant	608	Env 576→590	LQARVLAIERYLKDQ		X
	612	Env 580→594	VLAVERYLKDQKFLG		X
	624	Env 592→606	FLGLWGCSGKIICPT		X

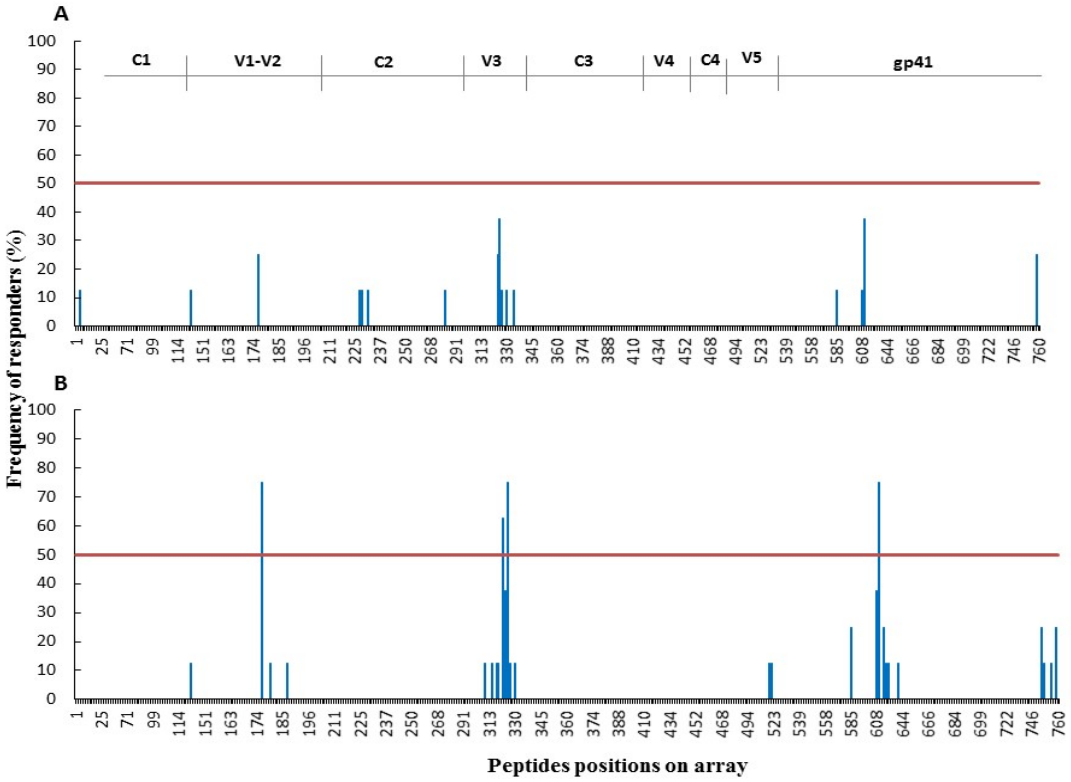

Figure 14. Frequency of antibody responses to HIV Env peptides in the vaccination groups. A and B show the frequency of antibody responses in low-dose group and high-dose group, respectively.

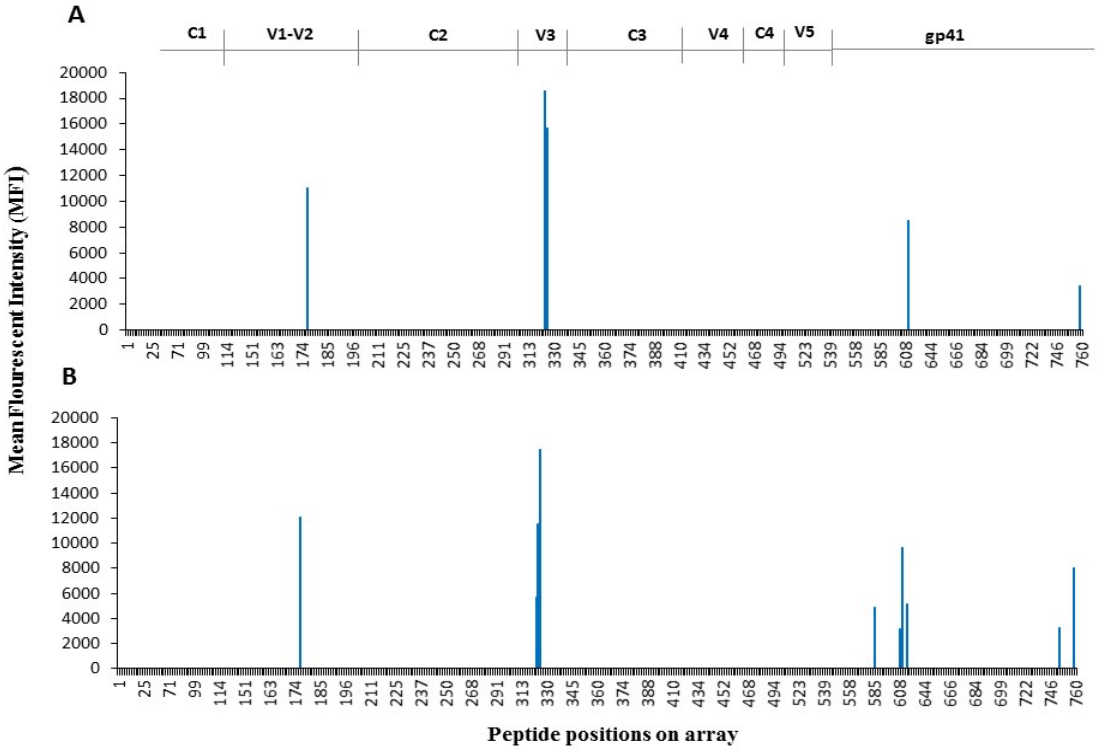

Figure 15. Shows the magnitude of antibody responses to HIV Env peptides in (A) low-dose group and (B) high-dose group.

6. DISCUSSION

6.1. Paper I

6.1.1. Suitability of the youth cohort in Maputo for phase I/II HIV vaccine trials

The overall prevalence of HIV (5.1%) in our study is similar to the prevalence of 4% reported in a study performed from November 2002 – April 2003 at the same youth clinic (112), but is lower than the national estimated prevalence of 10.9% for this age group in 2009. The free services offered by the youth clinic (HIV counselling and testing, STI diagnosis and treatment, condom provision and sexual behaviour education) could have contributed to the lower HIV-1 prevalence reported in both studies. Females had a significantly higher HIV prevalence (5.8%) as compared to males (3.1%), p = 0.018. Similar findings were previously reported in the country (15) and in other African countries (113; 114; 115), supporting the suggestion that women are more affected by the HIV-1 epidemic than men. The overall incidence of HIV-1 in our study was 1.14/100PY, which is lower compared to that found in previous studies conducted in the Mozambique, which indicated an HIV incidence in different groups that vary from 3.2 to 6.5 per 100 women years (WY) (116; 117; 118). In our study, all incident cases occurred in females, the HIV incidence rate among women was 1.49/100 WY (95% CI: 0.88–2.51). These findings further stress the need of additional intervention to protect young women from acquiring HIV infection. Retention rates in our study were similar to those reported by others (119) or even higher (120).

The high retention rates, relatively low prevalence and incidence of HIV in this cohort suggested that this group was suitable for a phase I/II HIV vaccine trials in our setting. Therefore, we recruited participants from this cohort to participate in phase I and II HIV vaccine trials in Mozambique, the TaMoVac I trial and TaMoVac II trial (not presented here), respectively.

6.1.2. HBV and syphilis prevalence, co-infections with HIV in young adults in Maputo City, Mozambique

The overall HBsAg prevalence in this study was 12.2%, this is higher than the previously reported prevalence in the country, 6.0% to 10.6% in blood donors (89; 90; 91) and 8% in women from a rural area of southern Mozambique (92). The majority of youths in Mozambique

never received Hepatitis B vaccinations. Hepatitis B vaccination was introduced in the Mozambican national program of immunization in 2001. Our study demonstrates that youths are severely affected by HBV infection, and therefore, vaccination and health education campaigns targeting adolescents and young adults should be considered. In this study, 11.3% of HIV-infected participants were also infected with HBV. Similar findings have been reported in other studies from Africa (121; 122). Since co-infections with these viruses are common, HIV-infected patients should benefit from HBV screening and vaccination.

The overall syphilis prevalence in our study was 0.36%, which was lower compared to that found in previous studies; 2.3% in the same population in volunteers recruited from 1 November 2002 to 31 April 2003 (112), and 2.2 % the national value in pregnant women in 2011 (123). Data from WHO indicates a trend of decline in rates of syphilis in Mozambique, from 7.9 % in 2008, 6.9% in 2009 to 5.7% in 2010, but the rate of infection is still high (72). In the present study, all participants with a reactive syphilis test had other sexually transmitted viral infections (HIV and/or HBV), suggesting that the route of transmission was likely to be the same and that infection with one STI increases the risk of acquiring other venereal infections (124). Therefore, further effort should be made to control syphilis and other STIs, which will also contribute to the prevention of HIV infections. In 2015, WHO launched the programme for validation of elimination of mother-to-child-transmission of HIV and syphilis. The success of this program will depend on the prevalence of disease in the country, health service coverage, and whether key populations with high transmission risk can access health services (125).

6.2. Paper II

6.2.1. Potential risk of HBV transmission from youths to their children or other unvaccinated persons

We found a high prevalence of HBV seromarkers (12.1% for HBsAg and 42.8% for Anti-HBc) and HBV incidence (180 per 100 000 PY using HBsAg and 2690 per 100 000 PY using Anti-HBc) in unvaccinated young adults in Maputo, Mozambique. Other studies conducted in Mozambique have also reported a high prevalence of markers of HBV infection, 6.0% to 10.6% for HBsAg (90; 92; 91; 89) and 63% to 64.5% for Anti-HBc (90; 92), confirming that HBV infections are endemic in the country. However, the prevalence of the HBsAg marker was much higher in our study than in previous studies, showing that the youth population are severely

affected by HBV infections. With regards to HBV incidence, previous studies conducted in Nigeria (126) and in Taiwan (127) have also reported a high incidence of this virus among youths.

Among the study participants, 8.5% were chronically infected with HBV. Besides the health problems that these youths may develop as a consequence of HBV infection, they constitute reservoirs of asymptomatic human carriers, who are potentially infectious. These youths may transmit HBV infections to their children or other unvaccinated persons. The high prevalence and incidence of HBV among youths stress the need for additional intervention to control HBV infection, especially among sexually active individuals. Vaccination for adolescents and youths who were not vaccinated during childhood is one example.

The prevalence of HBeAg in chronically infected females in our study was relatively low (6.5%), similar to what has been reported in other African countries (128; 129; 130). Despite the relatively low prevalence of HBeAg found in our study, the high prevalence (11.0%) and incidence (160 per 100 000 PY using HBsAg and 2560 per 100 000 PY using anti-HBc) of HBV infections among women of child bearing age, suggest that HBV vertical transmission may play important role in Mozambique, especially because neonatal immunization is not practiced in the country.

6.3. Paper III

6.3.1. Study derived reference values and comparison with those from other countries

In the present study, females had significantly higher values of absolute and percentage CD4+ cells count, and CD4:CD8 ratio than males. Our findings are similar with those reported for adults in Kericho, Kenya (131). Other studies have also reported higher CD4+ T cell counts among females than among males (132; 133; 134; 135; 136). The study reference ranges were generally comparable to those from the Western Kenya and North American populations. Generally, the lower and upper limits of the USA reference ranges for all T cells were higher than those derived from this study. The hematological values derived from this study, in most cases, were lower than those derived from the USA population. This is consistent with studies conducted in western Kenya and Uganda involving similar age groups (132; 67). For many parameters, the lower limits of the ranges generated in the present study were lower than those derived from those African countries. About 50% of the participants had Hb values that were

outside the lower limits of the US reference range. Several factors have been suggested to account for low Hb values, including poor nutritional status, genetic red blood cell disorders or parasitic infections. A study conducted in Mozambique showed that parasitic infections are common in country (137). The reference ranges for chemistry parameters derived from this study were lower compared to those from western Kenya in the same age group. The Maputo values were comparable to US values, with a few exceptions. The upper limits of the study reference ranges for ALT, AST, bilirubin and cholesterol were somewhat higher than those from the US, which is consistent with results from Uganda (65). The reasons for the variation of the laboratory reference values between populations include ethnic origin, genetics, gender, altitude and environmental factors (132; 138; 139).

6.3.2. Study derived reference values and implications for clinical trials

Notably, a large proportion of the study participants (62%) would have been excluded in potential clinical studies due to abnormal laboratory values, if USA ranges were used as inclusion and/or exclusion criteria to assess participant eligibility. The hematological and clinical chemistry parameters would have been the main cause for participant exclusion. However, if the local reference values were used, only 40 (16%) of participants would have been excluded. It has been reported that the use of appropriate reference values contributed to reducing the workload and the study cost, by shortening the time period of trial enrolment (65).

The DAIDS toxicity tables for grading adverse events (AEs), which have been used in many clinical trials, predicted 297 adverse events in the laboratory values obtained from the blood samples of the Maputo youths. These results are consistent with those reported in Uganda (65) and western Kenya (67), which also predicted a high number of AEs using the DAIDs tables. This suggests that the toxicity tables used in the intervention studies for safety evaluations may not be appropriate for the young adults included in the present study, because it might lead to over reporting of adverse events.

6.4. Optimizing the dose of the HIV-DNA prime vaccine - Paper IV

A previous trial conducted in Tanzania (HIVIS03), which used the same vaccination strategy, showed that priming intradermal with 1000μg of HIV-DNA followed by an intramuscular HIV-MVA boost induces broad and strong immune responses. The dose of 1000 μg of the HIV-DNA vaccine was administrated using the Bioject® needle-free device in 5 injections of 0.1ml and separating Env and Gag plasmid pools (55). Another trial, the TaMoVac 01 Tanzanian study, evaluated the possibility of simplifying the regime used in HIVIS03 to 2 injections, using separated and combined plasmids. However, the maximum dose that could be delivered intradermally with the Bioject needle free device in 2 injections was 600 μg, since this device can only contain 0.1ml while the highest concentration of HIV-DNA available was 3 mg/ml. Thus a strict comparison between 2 and 5 injections could not be made. Nevertheless, the results of that trial showed that the simplified regimen (2 intradermal injections of a total of 600 μg of HIV-DNA), either using separated and combined plasmids, have similar priming effect as the standard regimen (5 intradermal injections of a total of 1000 μg HIV-DNA) (140). In the present trial, we compared the safety, tolerability and immunogenicity of priming with 2 intradermal injections of a total dose of 600 μg, with 2 intradermal injections of a total dose of 1200 μg using the needle-free Zetajet injection device that allows up to 0.2 ml intradermal injections, followed by HIV-MVA vaccinations. This was the first study to assess HIV-DNA delivery using the Zetajet, in a volume of 0.2 ml intradermally. Overall, the vaccines were safe and well tolerated. A good safety profile of the use of this prime-boost vaccination strategy has been reported in previous trials (54; 55; 140).

This vaccination strategy, HIV-DNA prime and HIV-MVA boost, was highly immunogenic in Mozambican vaccinees, with the high dose inducing stronger cellular immune responses than the low dose. After the first HIV-MVA boost, IFN-γ ELIspot responses to Env were significantly higher in vaccinees that received the higher DNA dose compared to those that received the lower dose (median 157.5 vs. 420 SFC/million PBMC, p=0.014), and a trend towards a difference was observed when only responders were compared (median 150 vs. 420 SFC/million PBMC, p = 0.0513). In this trial, ELIspot responses to Gag and Env were higher after the first than after the second HIV-MVA boost as had been previously reported for this vaccine candidate (54; 140; 55) and by others using HIV-DNA subtype C and HIV-MVA (141). The lack of responses to the second HIV-MVA may be attributed to pre-existent immunity against the vector proteins as previously reported (142).

In the present study, binding antibodies were elicited in all vaccinees four weeks after the last HIV-MVA boost, similar to what we have previously reported (140; 55; 143; 57). Another trial that used a similar vaccination strategy reported a relatively lower frequency (75%) of binding antibodies (141). The HIV-MVA vaccine used in our study has been evaluated in a HIV-MVA-alone strategy in the USA and Thailand. After immunization with three doses of 10^8 pfu HIV-MVA, binding antibodies were elicited in 90-100% of participants (144).

Vaccine-induced IgG binding antibodies against at least one antigenic site in the V2 loop were detected in 8/16 (50%) of the vaccinees. The frequency of anti-V2 responders was significantly higher in the high-dose group than in the low-dose group, 6/8 (75%) vs. 2/8 (25%), respectively, P=0.0486. The high dose of HIV-DNA induced the highest average number of antigenic sites recognized. These results give further evidence that priming with a high dose of HIV DNA might be beneficial for priming humoral immune responses. Pre-clinical studies have suggested that Env-specific antibodies are essential for blocking acquisition of infection (43). In addition, in the RV144 clinical trial, which demonstrated an efficacy of 31.2%, the presence of anti V1/V2 binding IgG antibodies were associated with protection (61).

Overall, the results of this study suggest that priming with a high dose of HIV DNA (1200 µg) is safe and shows immunological advantage over the lower HIV-DNA dose (600 µg).

7. RECOMENDATIONS AND FUTURE PLANS

We established a youth cohort and characterized the potential of this group to participate in phase I /II HIV vaccine clinical trials. We determined the prevalence of HIV, HBV and syphilis, as well as the incidence of HIV and HBV, and risk factors related to the spread of these STIs in the group. The results of this study indicated that this group is potentially a suitable population for phase I/II HIV vaccine trials in Mozambique. However, additional intervention should be made to further reduce the risk of acquiring HIV infection in this group. The participants included in these studies were not representative of the general Mozambican population. Therefore, community-based studies should be encouraged to better estimate the prevalence and incidence of HIV, HBV and syphilis within the overall population in Mozambique.

Based on the high prevalence and incidence of HBV among young adults found in this study, we recommend the implementation of a HBV immunization program targeting this group and adolescents who were not vaccinated during their childhood. Additionally, we also showed that HBV/HIV co-infections are common among youths. Therefore, we recommend that HBV vaccination should be considered for HIV mono-infected patients to prevent HBV infections.

Since the present study documented a high prevalence of HBV among women of childbearing age, we suggest that routine HBsAg screening be introduced for all pregnant women during the antenatal period and the first HBV vaccine dose be given within 24 hours of birth, for at least new born babies of mothers found to be HBsAg positive, to prevent perinatal transmission.

Young adults, especially women, are severely affected by HIV infection in Mozambique. In the present study it has been shown, that once again, education plays an important role in HIV prevention. Therefore, we strongly recommend incorporating sexual education topics into the national education system that aim to reduce the risk of HIV infection and other STIs in adolescents and young adults.

Clinical laboratory reference values derived from populations living in Europe and North America have been used in Mozambique for patient management, due to the absence of locally derived values. The results of this study provided the first indication that reference values derived from a Mozambican population differ from those derived from the populations mentioned above. Therefore, local reference values should be established for patient management and for clinical studies in Mozambique.

TaMoVac 01 was the first HIV vaccine clinical trial conducted in Mozambique. Through this study we increased capacity in the laboratories and in the clinic site of INS to conduct vaccine trials. As a result of this, we successfully conducted a multicentre trial, called TaMoVac II, which also included participants from the youth cohort. Currently, the site is conducting a HIV incidence study in high-risk populations, in preparation for phase IIb and III vaccine trials.

We demonstrated that the delivery of HIV-DNA vaccine i.d. at concentration of 3 mg/mL, in a volume of 0.2 ml using the Zetajet™ intradermally was safe and highly tolerable in healthy Mozambican volunteers. Therefore, this study supports the use of the Zetajet™ for i.d. delivery of HIV-DNA. We have also shown the superiority of priming with the higher dose (1200 µg) of HIV-DNA to generate cell-mediated immune responses. We recommend a further large-scale study to confirm the results of the TaMoVac 01 Maputo.

The findings presented here will guide future development of the HIV-DNA/HIV-MVA vaccine concept.

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