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EVALUATION OF VACCINE CANDIDATES FOR HIV PREVENTION IN TANZANIA

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Evaluation of Vaccine Candidates for HIV Prevention in Tanzania

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my beloved wife, Siya.

POPULAR SCIENCE SUMMARY OF THE THESIS

Despite the remarkable success that has been achieved in the global fight against human immunodeficiency virus (HIV), the pace at which new HIV infections are falling is insufficient. A total of 1.5 million people worldwide acquired HIV infection in 2020. This shows that ending the HIV epidemic will require an intervention whose effectiveness does not depend on human behaviour or adherence. The results from the Thai HIV vaccine efficacy trial (RV144) provided the first evidence that vaccine-induced protection against HIV infection is possible. The binding of IgG antibodies to the gp70V1V2 protein of HIV-1 envelope (Env) was associated with reducing the risk of acquiring HIV-1 infection, whereas, the binding of IgA antibodies to Env was associated with increasing the risk.

Our primary goal was to investigate whether the antibody responses known to reduce the risk of HIV-1 infection were elicited in Tanzanian vaccine recipients primed with a DNA-based HIV vaccine candidate (HIV-DNA) and boosted with a poxvirus-based HIV vaccine candidate (HIV-MVA) with or without an envelope protein boost (CN54rgp140/GLA-AF). Additionally, we determined the impact of vaccine-induced HIV antibodies on the performance of rapid test-based HIV diagnostic algorithms, as well as the suitability of female sex workers (FSW) in Dar es Salaam for participation in HIV vaccine efficacy trial.

We found that the HIV-DNA and HIV-MVA vaccination strategy elicited antibody responses associated with decreasing the risk of HIV infection in the majority of Tanzanian vaccinees. Furthermore, the induced anti-V1V2 IgG responses exhibited remarkable durability, lasting for three years in 75% of the vaccinees. We also learned that addition of an Env protein (CN54rgp140/GLA-AF) boost does not enhance the anti-V1V2 responses elicited by the HIV-DNA/MVA regimen. HIV antibodies induced by vaccination significantly impact the accuracy of HIV diagnostic algorithms in sub-Saharan Africa, the region most affected by the HIV epidemic. The FSW in Dar es Salaam are suitable for enrolment in HIV vaccine efficacy trials. They have a high HIV incidence rate and it is possible to recruit and follow them over time, a prerequisite for inclusion into HIV vaccine trials.

In summary, priming with HIV-DNA followed by boosting with HIV-MVA elicits robust and durable anti-V1V2 responses in a majority of vaccinees. Boosting with a combination of CN54rgp140/GLA-AF and HIV-MVA does not augment the V1V2 response elicited by HIV-DNA priming and HIV-MVA boosting. The rapid test-based HIV testing algorithms in sub-Saharan Africa will misclassify a substantial proportion of HIV negative vaccine recipients as HIV positive. The FSW in Dar es Saalam are a suitable target population for HIV vaccine efficacy trials.

ABSTRACT

Background: This thesis describes the capability of an HIV-1 DNA and HIV-1 modified vaccinia virus Ankara (MVA) prime boost vaccination strategy to elicit immune responses known to correlate with reduced risk of HIV infection. Additionally, it describes the confounding effect of vaccine-induced HIV antibodies on the performance of HIV testing algorithms in sub-Saharan Africa, as well as the recruitment and preparation of a cohort of female sex workers (FSW) for potential participation in an HIV vaccine efficacy trial.

Methods: **Study I** evaluated the capability of the HIV-DNA/MVA vaccine regimen to elicit potent and durable binding antibody responses to the V1V2 domain of HIV-1 gp120. Plasma samples collected at peak immunogenicity and three years later, were analyzed for frequency, magnitude and persistence of antibodies to gp70V1V2 proteins. **Study II** investigated whether addition of an envelope protein (CN54rgp140/GLA-AF) boost would increase anti-V1V2 responses elicited by the HIV-DNA/HIV-MVA vaccine. **Study III** assessed the impact of vaccine-induced seroreactivity on the performance of rapid test-based HIV diagnostic algorithms. The HIV diagnostic strategies of Mozambique and Tanzania were evaluated using samples collected from vaccine recipients in the HIVIS and TaMoVac clinical trials. **Study IV** assessed the suitability of FSW in Dar es Salaam for participation in a phase IIb HIV vaccine efficacy trial (PrEPVacc). HIV incidence, retention rate and risk behaviours were determined in a cohort of 700 FSW after one year of follow up.

Results: Frequent and durable anti-V1V2 responses were elicited in the majority of the vaccine recipients. At peak immunogenicity, 97% of the vaccinees had binding IgG antibodies to the V1V2 loop of CRF01_AE A244. The anti-A244 V1V2 IgG persisted for at least three years in 75% of vaccinees and the response rate was improved by a late HIV-MVA boost given three years after the second boost (**study I**). The CN54rgp140/GLA-AF boost did not enhance the V1V2 response (**study II**). The HIV diagnostic algorithms in sub-Saharan Africa misdiagnosed a substantial proportion of HIV vaccine recipients. More than half (54%) of the vaccinees would have been incorrectly identified as HIV infected in Tanzania, while, 26.3% would have been misclassified in Mozambique (**study III**). A high HIV incidence of 3.45 per 100 person years at risk was observed among FSW in the PrEPVacc preparedness study. The rate of HIV acquisition was higher among the young (18-24 years), drug using FSW, and those with syphilis or hepatitis infections (**study IV**).

Conclusion: Priming with HIV-DNA followed by boosting with HIV-MVA elicited robust and durable anti-V1V2 responses in a majority of vaccinees. Boosting with a combination of CN54rgp140/GLA-AF and HIV-MVA did not augment anti-V1V2 responses elicited by HIV-DNA priming and HIV-MVA boosting. The current HIV testing algorithms in sub-Saharan Africa cannot sufficiently discriminate vaccine-induced seroreactivity from true HIV infection. Young FSW in Dar es Saalam are a suitable target population for HIV vaccine efficacy trials.

LIST OF SCIENTIFIC PAPERS

- I. Joachim A, **Msafiri F**, Onkar S, Munseri P, Aboud S, Lyamuya EF, Bakari M, Billings E, Robb ML, Wahren B, Mhalu FS, Sandström E, Rao M, Nilsson C, Biberfeld G. Frequent and Durable Anti-HIV Envelope VIV2 IgG Responses Induced by HIV-1 DNA Priming and HIV-MVA Boosting in Healthy Tanzanian Volunteers. *Vaccines*. 2020;8(4):681.
- II. **Msafiri F**, Joachim A, Held K, Nadai Y, Chisumba RM, Geldmacher C, Aboud S, Stöhr W, Viegas E, Kroidl A, Bakari M, Munseri PJ, Wahren B, Sandström E, Robb ML, McCormack S, Joseph S, Jani I, Ferrari G, Rao M, Biberfeld G, Lyamuya E, Nilsson C. Frequent Anti-V1V2 Responses Induced by HIV-DNA Followed by HIV-MVA with or without CN54rgp140/GLA-AF in Healthy African Volunteers. *Microorganisms*. 2020;8(11):1722.
- III. **Msafiri F**, Manjate A, Lindroth S, Tembe N, Matavele R, Cumbane V, Jani I, Aboud S, Lyamuya E, Andersson S, Nilsson C. Vaccine-Induced Seroreactivity Impacts the Accuracy of HIV Testing Algorithms in sub-Saharan Africa. An exploratory study (*Manuscript*)
- IV. Faini D*, **Msafiri F***, Munseri P, Bakari M, Lyamuya E, Sandstrom E, Biberfeld G, Nilsson C, Hanson C, Aboud S. The prevalence, incidence and risk factors for 1 HIV among female sex workers – a cohort being prepared for a phase IIb HIV vaccine trial in Dar es Salaam, Tanzania. (*Manuscript*)
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LIST OF ABBREVIATIONS

| | |
|---------|---|
| ADCC | Antibody-Dependent Cellular Cytotoxicity |
| ADCD | Antibody-Dependent Complement Deposition |
| ADCP | Antibody-Dependent Cellular Phagocytosis |
| ADNP | Antibody-Dependent Neutrophil Phagocytosis |
| AIDS | Acquired Immunodeficiency Syndrome |
| ART | Antiretroviral Therapy |
| C2 | Constant region 2 |
| C4 | Constant region 4 |
| CMDR | Chiang Mai Double Recombinant |
| DNA | Deoxyribonucleic Acid |
| ELISA | Enzyme Linked Immunosorbent Assay |
| Env | HIV envelope |
| FSW | Female Sex Workers |
| GLA-AF | Glucopyranosyl Lipid Adjuvant - Aqueous Formulation |
| HIV | Human Immunodeficiency Virus |
| IFN | Interferons |
| IL | Interleukins |
| NIAID | National Institute of Allergy and Infectious Diseases |
| NIH | National Institute of Health |
| MIP | Macrophage Inflammatory Protein |
| MVA | Modified Vaccinia Virus Ankara |
| PrEP | Pre-Exposure Prophylaxis |
| PYR | Person Years at Risk |
| RDS | Respondent Driven Sampling |
| RDT | Rapid Diagnostic Test |
| RNA | Ribonucleic Acid |
| SIV | Simian Immunodeficiency Virus |
| TAF/FTC | Tenofovir Alafenamide/Emtricitabine |
| TDF/FTC | Tenofovir Disoproxil Fumarate/Emtricitabine |
| TNF | Tumour Necrosis Factor |

| | |
|------|--------------------------------|
| VISP | Vaccine Induced Seropositivity |
| VISR | Vaccine Induced Seroreactivity |
| V1V2 | Variable regions 1 and 2 |
| V3 | Variable region 3 |
| WB | Western Blot |

1 INTRODUCTION

1.1 DISCOVERY OF HUMAN IMMUNODEFICIENCY VIRUS (HIV)

The world first became aware of acquired immunodeficiency syndrome (AIDS) in the summer of 1981 when a number of young homosexual men were diagnosed with cellular immune dysfunction.^{1,2} Most of these patients later succumbed to rare opportunistic infections such as *Pneumocystis carinii* pneumonia and Kaposi's sarcoma.³⁻⁵ In 1983, a T-lymphotropic retrovirus was isolated from a patient at risk for AIDS,⁶ and was subsequently confirmed as the causative agent of AIDS.⁷⁻⁹ The retrovirus was officially named human immunodeficiency virus (HIV) in 1986.¹⁰ HIV is a consequence of multiple cross-species introductions of simian immunodeficiency virus (SIV).^{11,12} HIV-1 and HIV-2 are close relatives of SIV found in chimpanzees¹³ and sooty mangabeys¹⁴ respectively.

1.2 THE GLOBAL HIV EPIDEMIC

More than 75 million people have contracted HIV since the start of the epidemic, and regrettably, over 32 million have lost their lives to AIDS-related illnesses.¹⁵ However, tremendous progress has been achieved in the global response to HIV. Of the estimated 37.7 million people living with HIV in 2020, 84% were aware of their HIV status, 87% of those knowing their HIV-positive status were on treatment, and 90% of those on antiretroviral therapy (ART) were virally suppressed. Moreover, increased access to ART has saved about 16.6 million lives globally in the past two decades, resulting in a 47% reduction in AIDS-related mortality since 2010.¹⁶ Despite the remarkable success, the progress in decreasing new HIV infections remains inadequate. In 2020, a total of 1.5 million people became newly infected with HIV worldwide, with 60% of the infections occurring in sub-Saharan Africa. Furthermore, no significant decline in the number of new HIV infections has been observed among adults since 2018. Globally, the target of reducing adult HIV infections by 75% in the last decade was missed by a huge margin, with infections dropping by 31% only since 2010.¹⁶

Key populations and their sexual partners drive the HIV epidemic in adults. They accounted for nearly two-thirds (65%) of all HIV infections in 2020 and 93% of the HIV incidence outside of sub-Saharan Africa. Criminalization and marginalization undermine efforts to curb the spread of HIV in key populations.¹⁶ Similarly, gender inequality and discrimination continue to increase the risk of HIV acquisition among women.

In sub-Saharan Africa, 25% of HIV infections in 2020 occurred among young women (15-24 years) and adolescent girls, despite them making up just 10% of the population.¹⁶ While the HIV epidemic expanded in eastern Europe, central Asia, Middle East and North Africa in the past decade, a sharp decline was observed in eastern and southern Africa where HIV infections in adults and children fell by 43% and 64% respectively, since 2010. The region managed to reduce the spread of HIV despite accounting for 55% of the global HIV burden and two-thirds of all HIV infections in children.¹⁶

1.3 THE HIV EPIDEMIC IN TANZANIA

At the end of 2020, an estimated 1.7 million Tanzanians were living with HIV, equivalent to a prevalence of 4.7%.¹⁷ By 2018, 78% of those living with HIV knew they were infected, 92% of those knowing their infection status were on ART, and 87% of those receiving treatment were virally suppressed.¹⁸ Women in Tanzania continue to be disproportionately affected by the epidemic. They accounted for 59% of the HIV burden in 2020, with the HIV prevalence among young women being twice as high compared to young men. Moreover, of the 58,000 adults who acquired HIV in 2020, nearly two-thirds (64%) were women.¹⁷ The disparity between the sexes is largely driven by gender inequality and sexual violence.^{19,20}

The prevalence of HIV among key populations in Tanzania remains unacceptably high. At the end of 2018, 15.4% of female sex workers (FSW), 15.5% of people who inject drugs and 8.4% of men who have sex with men (MSM) in Tanzania were living with HIV.^{17,21} Sexual partners of these high-risk groups serve as a “bridging population” transferring the infection from key populations to the general population.²² Despite the challenges, Tanzania has made significant gains over the last decade. The number of new HIV infections and AIDS-related mortality has decreased by 13% and 50% respective, since 2010.¹⁵

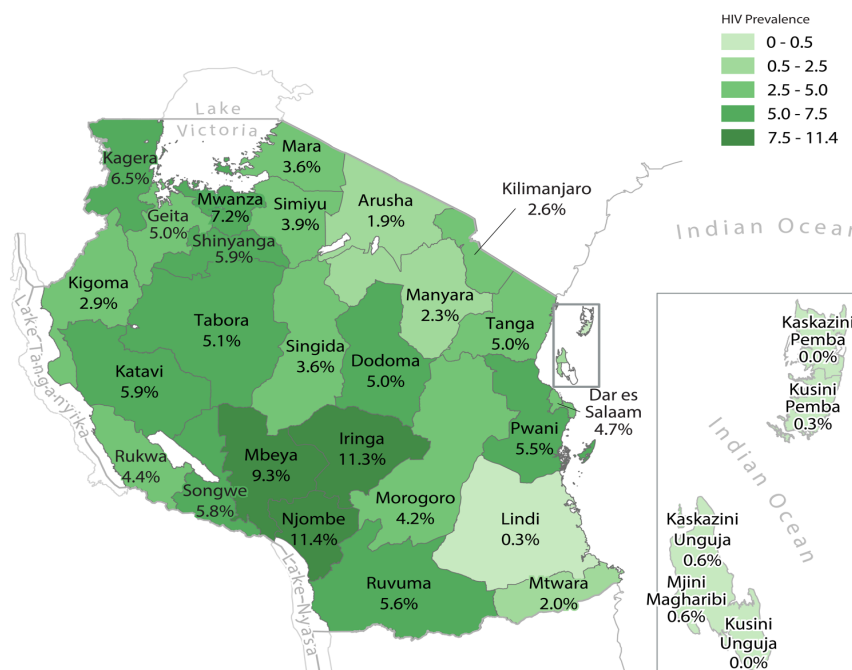


Figure 1. HIV prevalence by regions in Tanzania.

Source: Tanzania HIV impact survey (THIS) 2016-2017.²³

1.4 THE NEED FOR AN HIV VACCINE

Despite the success of existing HIV prevention methods in reducing AIDS associated mortality and new HIV infections, transmission continues to occur. Globally, 1.5 million people were infected with HIV in 2020. Moreover, the number of new HIV infections among adults has remained static in recent years.¹⁶ Modeling studies predict that an HIV-1 vaccine with a 50% efficacy will avert millions of new infections and substantially slow the pandemic.²⁴

Highly active antiretroviral therapy (HAART) does suppress viral replication but cannot prevent establishment of latent viral reservoir or eradicate them. Therefore, lifelong persistent HIV infection ensues in infected individuals, even if they are put on HAART.^{25,26}

To continue keeping the HIV epidemic under control, the 27.5 million people currently receiving HAART, must be kept on care and treatment for the rest of their lives. Treatment must also be extended to the 10.2 million people who are infected but not yet on anti-retroviral drugs, and to those who become newly infected with HIV every year. All this translates to an increase in global resources required for responding to the HIV epidemic. To end AIDS as a public threat to health by 2030, the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that the annual HIV budget in low- and middle-income countries will need to rise to US\$ 29 billion.¹⁶ The high financial cost being incurred in the fight against the HIV epidemic may not be sustainable in the future. An effective HIV vaccine will provide a sustainable, cost effective, long-term solution to ending the HIV epidemic.²⁷

Finally, sustaining the current gains without vaccination will be difficult, as human behavior is unpredictable. Thus, for a durable end to the HIV epidemic, we need a biomedical intervention whose effectiveness will not depend on individual adherence.²⁸

1.5 BARRIERS TO HIV-1 VACCINE DEVELOPMENT

A vaccine is needed to end the HIV and AIDS pandemic.²⁹ However, the lack of knowledge on immune responses required for protection, the rapid establishment of latent viral reservoirs, the extraordinary genetic diversity of HIV-1, the inability to elicit HIV-1 broadly neutralizing antibodies, and the capability of the virus to escape host immune responses represent formidable hurdles to the development of prophylactic HIV-1 vaccines.³⁰

Natural infection does not induce immunity against HIV-1. Since patients are unable to clear the virus, it remains unclear which immune correlates should be elicited to confer protection.³⁰

The HIV-1 group M is comprised of nine clades and 118 CRFs.^{31,32} Mutations induced by error-prone reverse transcriptase combined with the high replication rate give rise to the extraordinary diversity of HIV-1. Variations in the amino acid sequences of HIV-1 Env can be as high as 20% within a clade and over 35% between clades.^{33,34} An effective vaccine will need to confer cross protection against most of the circulating clades and recombinants.

Most licensed viral vaccines afford protection through generation of broadly neutralizing antibodies. Antibody access to conserved epitopes in the HIV-1 Env glycoprotein is effectively blocked by the dense and poorly immunogenic N-linked glycosylation shields.^{35,36} Furthermore, the formation of chemokine co-receptor binding site only occurs after HIV-1 Env undergoes extensive conformational change upon binding to its cellular receptor.³⁷ Additionally, mutations in N-linked glycans enhance the ability of the virus to evade the host's neutralizing antibodies.^{38,39} It is also difficult to target the membrane proximal external region (MPER) of gp41 by vaccination because of sequestration of the epitope in the lipid membrane,⁴⁰ tolerance control and immunoregulation,⁴¹ or transient exposure of the MPER during viral entry.⁴² Moreover, the B cell precursors of broadly neutralizing antibodies are very rare or difficult to activate because of autoreactivity and the presence of unusual traits such as rare somatic mutations or long third heavy chain complementary determining regions (HCDR3s) in HIV-1 broadly neutralizing antibodies.^{43,44} However, antibodies capable of neutralizing the CD4 binding site have been detected in a small proportion of HIV-1 infected individuals.⁴⁵

Although HIV-1 specific CD8⁺ T lymphocytes have been associated with control of primary viraemia⁴⁶⁻⁴⁸ and maintenance of stable viral load,⁴⁹ vaccine-induced cellular responses are less likely to prevent lifelong infection since the virus establishes latent reservoirs within the first 10 days of infection.^{50,51}

1.6 CORRELATES OF DECREASED RISK OF HIV INFECTION

A major goal for HIV-1 vaccine efficacy trials is to identify the immune responses that decrease the risk of acquisition of HIV-1 or suppress viral replication if infection occurs.⁵² A number of immune correlates were associated with low risk of acquiring HIV in VAX004, RV144, and HVTN 505 efficacy trials.

The Vax004 trial evaluated protein immunogens with an aim of inducing antibody responses against the HIV-1 Env.⁵³ Despite lacking vaccine efficacy, neutralizing antibodies to easy to inactivate viruses (Tier 1) and antibody dependent cellular virus inhibition (ADCVI) activity correlated with low risk of HIV-1 infection in the Vax004 vaccinees.⁵⁴

The HVTN 505 efficacy trial tested the DNA/Ad5 vector (Clade A, B, C, Env, Clade B Gag/Pol) in circumcised MSM and transgender participants.⁵⁵ Although the trial was halted for futility, the elicited polyfunctional CD8⁺ T cell responses were significantly associated with reducing the risk of acquiring HIV-1.⁵²

The Step (HVTN 502) trial aimed at evaluating the capacity of HIV-1 specific CD8⁺T cells to suppress viral replication in breakthrough infections.⁵⁶ Despite identifying subdominant HIV-1 specific CD8⁺ T cell responses with antiviral activity in a subset of vaccinees, subsequent virus sieve analysis revealed that the T cell responses were not elicited by vaccination.⁵⁷

Two primary immune correlates of risk were identified in the RV144 trial, the first HIV-1 vaccine trial to demonstrate efficacy.⁵⁸ A modest vaccine efficacy of 31.2% was observed in vaccinees after 3.5 years of follow up.⁵⁹ Binding IgG responses to the variable regions 1 and 2 (V1V2) of the HIV-1 Env correlated with decreased risk of HIV infection,^{58,60} while plasma IgA responses to HIV-1 Env were associated with increasing the infection risk.^{58,61} Secondary analysis revealed that vaccine-elicited IgG3 antibody responses to HIV-1 Env inversely correlated with infection risk.⁶² Moreover, antibody-dependent cellular cytotoxicity (ADCC)-mediating antibodies correlated with reduced HIV-1 infection risk in the presence of low levels of vaccine-elicited plasma IgA responses.^{63,64} A summary of the HIV-1 vaccine efficacy trials is provided in Table 1.

Table 1. Summary of HIV-1 vaccine efficacy trials

| Study | Vaccines | Phase | Volunteers' risk | Location | Results | Correlates of decreased HIV risk |
|-------------------------|---|-----------|---|--|---|--|
| Vax004 | AIDSVAXR_ B/B gp120 in alum | III | MSM and women at high risk | United States, Europe | No efficacy | ADCVI, CD4 Blocking, Tier 1 NAb |
| Vax003 | AIDSVAXR_ B/E gp120 in alum | III | Injecting drug users | Thailand | No efficacy | None |
| HVTN 502 Step trial | MRKAd5 HIV-1 gag/pol/nef B | I Ib | MSM and heterosexual women and men | United States | Stopped early. No efficacy; transient increased infection rate in vaccinees | None |
| RV144 | ALVAC-HIV (vCP1521) and AIDSVAXR_ B/E rgp120 in alum | III | Community-risk population | Thailand | 31.2% efficacy at 42 months, 60% at 12 months against HIV acquisition. No effect on plasma viral load and CD4 count | V1V2 IgG, Linear V2, V1V2 IgG3, Interactions (ADCC, Avidity, Tier 1 NAb, IgA), CD4 ⁺ T cell Polyfunction, Cytokines |
| HVTN 503 Phambili trial | MRKAd5 HIV-1 gag/pol/nef B | I Ib | Heterosexual men and women | Republic of South Africa | No efficacy; increased HIV infection rate in vaccinees | n/d |
| HVTN 505 | DNA and rAd5 (A, B, and C) | I Ib | MSM with Ad5-specific antibody titers <1:18 (negative | United States | Stopped for fertility; no efficacy on HIV acquisition, plasma viral load and CD4 count | CD8 ⁺ Env T-cell |
| HVTN 702 | ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59 | I Ib/ III | At risk adults | Republic of South Africa | Halted for lack of efficacy. Higher HIV incidence in vaccinees than in placebos | n/d |
| HVTN 705* | Ad26.Mos4.HIV + Clade C gp140 | I Ib | At risk women | South Africa, Malawi, Mozambique, Zambia, Zimbabwe | No sufficient protection against HIV. An efficacy of 25.2% (95% CI; -10.5% to 49.3%) was observed | n/d |
| HVTN 706 | Ad26.Mos4.HIV and adjuvanted clade C gp140 and Mosaic gp140 protein | III | MSM and transgender persons | Europe, North America and South America | Currently ongoing | |
| PrEPVacc | DNA/AIDSVAX and DNA/CN54gp140 + MVA/CN54gp140) with PrEP | I Ib | At risk adults | South Africa, Tanzania, and Uganda | Currently ongoing | |

Adapted from Kim JH, et al. 2015,⁶⁵ Tomara GD, et al. 2017,⁵² Ng'uni T, et al. 2020,⁶⁶ and Laher F, et al. 2020.⁶⁷ n/d; not done

* Results available at <https://www.jnj.com/johnson-johnson-and-global-partners-announce-results-from-phase-2b-imbokodo-hiv-vaccine-clinical-trial-in-young-women-in-sub-saharan-africa>

1.7 HIV TESTING IN SUB-SAHARAN AFRICA

HIV diagnosis in sub-Saharan Africa relies primarily on rapid diagnostic tests (RDTs).⁶⁸

To improve the accuracy of the results, the World Health Organization recommends the use of two or three RDTs either in series or in parallel.⁶⁸ As of January 2018, Determine™ HIV-1/2, SD Biotline HIV1/2, or Uni-Gold™ HIV-1/2 were the most utilized HIV-RDTs in Africa. (See Table 2 below).⁶⁹⁻⁸³

Table 2. HIV-RDTs commonly used in African countries

| HIV-RDT | Company | Countries reporting use |
|-----------------------------------|---|---|
| Determine™ HIV-1/2 | Alere Medical Co., Chiba, Japan | Uganda, Kenya, Guinea, Cameroun, DRC, Nigeria, Zambia, Malawi, South Africa, Rwanda, Burundi, Zimbabwe, Swaziland, Mozambique |
| Uni-Gold™ HIV-1/2 | Trinity Biotech, Bray, Ireland | Ethiopia, Uganda, Kenya, DRC, Nigeria, Zambia, Malawi, South Africa, Rwanda, Swaziland, Mozambique, Tanzania, Botswana |
| SD Biotline HIV1/2 3.0 | Standard Diagnostics Inc, Giheung-gu, Korea/Alere | Zambia, South Africa, Rwanda, Tanzania |
| HIV (1+2) Antibody colloidal Gold | KHB, Shanghai Kehua Bio-Engineering Co Ltd, China | Ethiopia, Rwanda, Botswana |
| HIV-1/2 STAT-PAK | Chembio Diagnostics, Medford, NY, USA/Alere | Ethiopia, Uganda |
| HIV1/2 Stat-Pak Dipstick | Chembio Diagnostics, Medford, NY, USA | Uganda, Nigeria |
| Immunoflow HIV1-HIV-2 | Core Diagnostics, UK | Guinea |
| Clearview COMPLETE HIV-1/2 | Chembio Diagnostics, Medford, NY, USA | Swaziland |
| Genie II HIV1/HIV-2 | BioRad, France | Burundi |
| First Response HIV 1-2.0 | Premier Medical Corporation, Daman, India | Zimbabwe |
| INSTI HIV1/HIV2 | Biolytical, Richmond BC, Canada | Zimbabwe |
| ImmunoComb HIV-1&2 BiSpot | Orgenics/Alere, Israel | Cameroun |
| ImmunoComb II HIV 1&2 CombFirm | Orgenics/Alere, Israel | DRC, Uganda |

1.8 ENROLMENT OF FEMALE SEX WORKERS IN HIV VACCINE EFFICACY TRIALS

Sex workers, people who inject drugs, prisoners, transgender people, gay men and other men who have sex with men are highly vulnerable to HIV infection due to social exclusion, violence and criminalization.⁸⁴ Globally, FSW are 26 times more likely to acquire HIV than other adult women.¹⁶ Given that FSW are highly mobile,⁸⁵ and experience a lot of stigma and discrimination at public health facilities,⁸⁶ enrolling them in an HIV vaccine preparatory study provides an opportunity to learn about the extent to which they will comply with required clinic visits and immunization procedures in the vaccine efficacy trials. Moreover, it informs strategies to improve recruitment and retention of FSW, especially the young ones who have high dropout rates.^{87,88} The loss of these participants may impact vaccine efficacy estimates in a randomized clinical trial if an imbalance in HIV exposure risk develops between the arms of a clinical trial.⁸⁹ Additionally, considering their high HIV infection risk, FSW will clearly benefit from participating in HIV vaccine efficacy trials, particularly if the vaccine regimen proves successful. Also, the high HIV incidence among FSW may lead to early demonstration of vaccine efficacy or failure minimizing trial duration and costs.⁸⁸

2 LITERATURE REVIEW

2.1 HIV STRUCTURE

The mature HIV virion is spherically shaped, 100–120 nm in diameter. It is surrounded by a protective lipid bilayer as its envelope. The virus contains spikes that protrude through the lipid membrane, and each spike consists of three glycoprotein (gp) 120 molecules non-covalently bound to transmembrane gp41. To the inside of the viral lipoprotein membrane, is a matrix protein (p17) and the cone-shaped nucleocapsid (core). The viral core consists of two identical copies of single-stranded RNA molecules combined with a nucleoprotein and the enzymes reverse transcriptase, integrase and protease.⁹⁰⁻⁹²

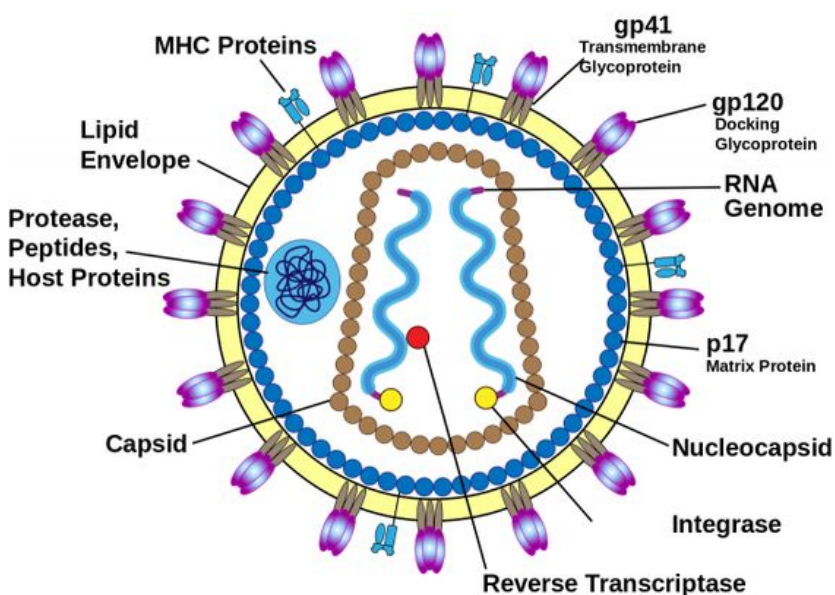


Figure 2. Structure of HIV

Source: <https://www.toppr.com/ask/question/explain-the-structure-of-hiv/>

2.2 THE HIV-1 GENOME

The HIV-1 genome contains two single-stranded RNA molecules enclosed within the viral core. The viral RNA molecules undergo reverse transcription into double-stranded DNA that gets incorporated into the human genome. The HIV DNA then encodes genes for the viral structural and regulatory proteins. The main structural genes are the gag, pol and env. The gag encodes for the matrix (MA, p17), capsid (CA, p24), and nucleocapsid (NC, p7) proteins, whereas, the pol encodes for the precursor of viral enzymes protease (PR, p12), reverse transcriptase (RT, p51), RNase H (p15) or RT plus RNase H (p66) and integrase (IN, p32). The protease cleaves the Gag polyprotein to produce structural proteins, whereas, reverse transcriptase transcribes DNA from RNA, and integrase mediates insertion of the DNA into the host chromosome. The env gene encodes for the large gp160 precursor molecule, which is cleaved by the viral protease to form surface glycoproteins gp120 and gp41.^{90,92,93}

In addition to structural proteins, the HIV DNA also encodes genes for regulatory proteins. These are Tat, Rev, Nef, Vpr, Vif and Vpu genes. The Tat gene promotes transcription of

HIV genes, whereas, Rev facilitates the export of viral mRNA from the nucleus to the cytoplasm. The Nef gene down regulates the expression of CD4 receptors in the plasma membrane to avoid interaction with the newly synthesized gp120 protein, while vif enhances the infectivity of progeny virus particles. The Vpr is believed to be involved in the arrest of the cell cycle and insertion of the provirus DNA into the nuclei of non-dividing cells, whereas, Vpu is required for successful release of the virions from infected cells. The Vpx gene is found in HIV-2 and performs the same function as Vpr in HIV-1.^{90,92,93}

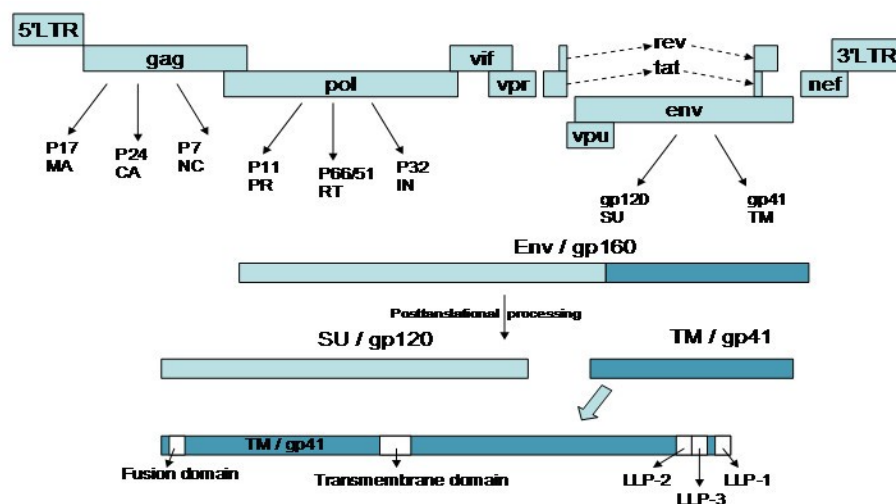


Figure 3. Organization of the HIV-1 genome

Source: <https://virologyj.biomedcentral.com/articles/10.1186/1743-422X-4-100/figures/3>

2.3 REPLICATION OF HIV-1

The entry of HIV-1 into target cells begins with the binding of HIV-1 envelope (Env) gp120 to the cellular receptor, CD4, on the surface of CD4+ cells, such as helper T-lymphocytes. Upon binding the CD4 receptor, the HIV-1 Env undergoes extensive conformational change that expose the chemokine co-receptor binding site, allowing the virus to interact with a cellular chemokine co-receptor CCR5 or CXCR4. The N-terminal fusion peptide of the transmembrane gp41 is then inserted into the host cell membrane resulting into fusion of the viral and cellular membranes. Following membrane fusion, the viral core is released into the cytoplasm of the host cell, freeing the viral RNA. The viral RNA genome is then reverse transcribed into a full-length double-stranded DNA (cDNA) by the viral reverse transcriptase. Formation of the viral cDNA occurs within a pre-integration complex composed of the matrix protein, Vpr and integrase. The pre-integration complex is then transported to the nucleus where the viral cDNA is integrated into the host chromosome, resulting in the formation of the provirus. Upon cell activation, expression of the genes in the HIV provirus occurs, leading to generation of the messenger RNA (mRNA). The viral messenger RNA migrates into the cytoplasm where structural proteins of the new virions are synthesized. The proteins encoded by pol and gag genes form the core, the capsid and matrix of the new viral particle, whereas, those encoded by the env gene form the envelope glycoproteins gp 120 and

gp41. Then, matured viral particle buds through the host cell membrane, acquiring a new envelope as it exits the cell.^{90,93,94}

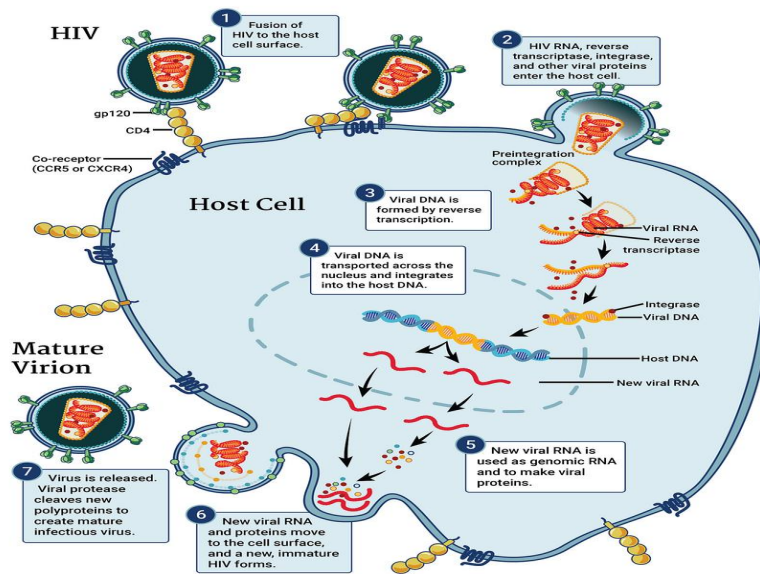


Figure 4. Replication cycle of HIV-1

Source: <https://www.niaid.nih.gov/diseases-conditions/hiv-replication-cycle>

2.4 HIV-1 DIVERSITY

The extraordinary global diversity of HIV-1 is driven by the high mutation rate introduced by the error-prone reverse transcriptase, high replication rate of the virus, and recombination resulting from co-infection of cells with two or more different HIV-1 strains.^{95,96} Strains within the same subtype may vary by up to 20%, whereas, variation between subtypes may be as high as 35%.³⁴ Based on sequence homology, HIV is classified into two types; HIV-1, which is responsible for 95% of all global infections, and HIV-2, the less virulent form.^{97,98} HIV-1 is subdivided into four groups; M (Major), O (Outlier), N (non-M, non-O) and P.³² HIV-1 group M is the pandemic form and is comprised of nine subtypes i.e. A to D, F to H, J and K. Additionally, there are circulating recombinant forms (CRFs), derived from recombination between different HIV subtypes. To date, 118 HIV-1 CRFs have been identified.³¹

HIV-1 subtype C accounts for 47% of all global infections and is the dominant subtype in southern Africa. In east Africa, the epidemic is dominated by subtype A, though there is a significant contribution by subtypes C and D. The majority of HIV-1 infections in west and central Africa are caused by CRF02_AG, while subtype B predominates in north Africa.^{32,99,100} Infections in the Americas, Caribbean, Australia, and western and central Europe are dominated by subtype B while subtype A drives the epidemic in eastern Europe.^{32,101-104} Asia is a “hotbed” of recombinant forms. CRF01_AE predominates in southeast Asia and east Asia, while subtype C is prevalent in south Asia (India).^{32,101} HIV-1 groups O, N, and P infections are uncommon and remain restricted to west and central Africa.^{11,105}

HIV-2 is largely confined to west Africa, although infections have been reported in Europe (Portugal and France), India and the United States of America. It is comprised of nine groups (A to I), with a majority of current infections being caused by groups A and D.¹⁰⁶

The extraordinary diversity of HIV-1 subtypes poses an enormous challenge to the development of an effective vaccine against HIV.¹⁰⁷ A successful vaccine must confer universal protection against most HIV-1 subtypes and recombinants. To achieve this, vaccine immunogen sequences must match the sequence diversity of viral strains circulating in the world. However, HIV-1 subtype distribution is subject to change due to migration and population mixing. The constant viral evolution may decrease the efficacy of a potential vaccine.^{108,109} Continuous surveillance of HIV-1 subtypes will be needed to improve vaccine efficacy. Furthermore, the evolving subtypes may necessitate periodic changing of an approved HIV vaccine (like for influenza viruses).¹¹⁰ The extensive genetic variability may also impact control and treatment of HIV due to emergence of variants that are more transmissible and more resistant to drugs.¹¹¹

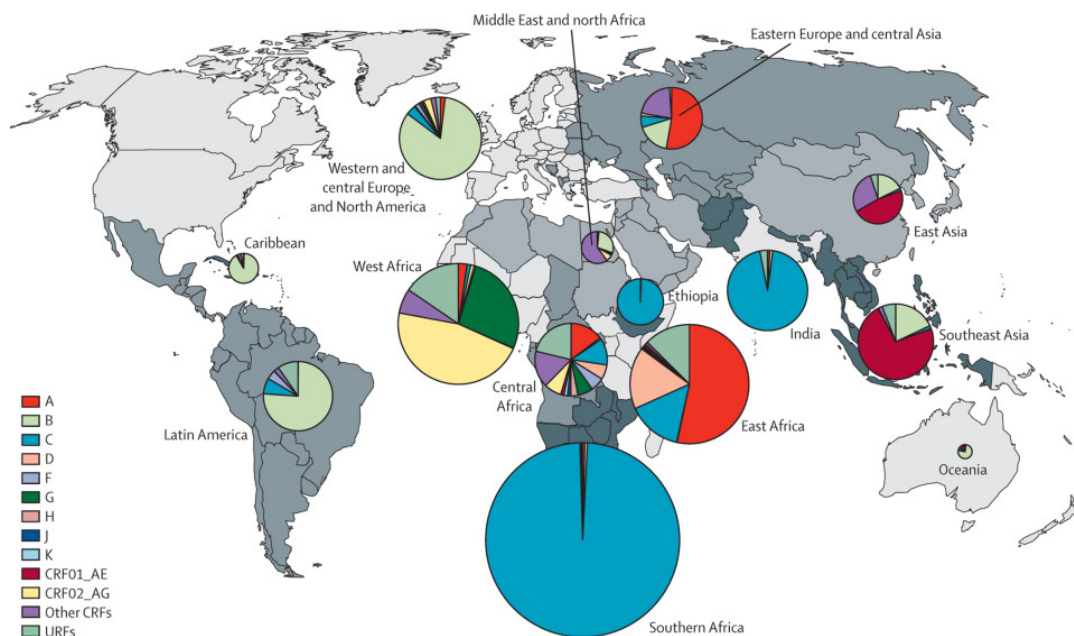


Figure 5. Global distribution of HIV-1 subtypes (Hemelaar J, et al. 2019).³² The figure has been reproduced with permission from the Lancet infectious diseases journal (reference no; 220328-017969).

2.5 THE IMMUNE RESPONSE TO HIV-1 INFECTION

2.5.1 Transmission of HIV-1

The majority of the HIV-1 infections occur through sexual transmission.¹¹² Nearly 80% of sexually acquired HIV-1 infections arise from a single transmitted virus.^{113,114} In the absence of a physical damage to mucosal epithelium, the exact mechanism by which the HIV-1 founder virus crosses the genital mucosa remains unclear. The virus may cross the epithelial barrier by transcytosis, or by direct contact with intraepithelial dendritic cells (DCs) or by passing through the intercellular spaces in the epithelium.^{112,115,116} Resident memory T cells expressing CD4 receptor and chemokine co-receptor 5 (CCR5) are usually the first to be infected.¹¹⁷ The early innate immune responses may inadvertently support propagation of the virus by recruiting activated CD4⁺ T cells and macrophages to the site of infection.¹¹⁸ The virus then spreads to the regional draining lymph node, where it infects more activated CD4⁺ CCR5⁺ T cells.¹¹² From the regional lymph nodes, HIV spreads to the gut-associated lymphoid tissue (GALT), which is rich in activated CD4⁺ CCR5⁺ memory T cells.^{119,120} Approximately 80% of CD4⁺ T cells in the GALT are lost within the first three weeks of HIV-1 infection, 20% as a result of direct infection, and 60% due to apoptosis caused by immune activation.^{119,121-123} Furthermore, the virus establishes latently infected reservoirs in lymphatic tissues within the first 10 days of HIV infection.⁵⁰

2.5.2 The innate response to HIV-1 infection

A surge in plasma levels of acute phase proteins is the first detectable systemic immune response to HIV-1. Increased levels of serum amyloid are seen when the virus is replicating in the genital mucosal tissues and draining lymph nodes.¹¹² Viraemia coincides with production of cytokines and chemokines. As viraemia increases, so do the concentrations of type I interferons (IFNs), CXC-chemokine ligand 10 (CXCL10), interleukin-15 (IL-15), IL-18, TNF, IFN γ , IL-22 and IL-10.¹²⁴ These pro-inflammatory cytokines and chemokines mediate apoptosis of activated DCs and the bystander destruction of CD4⁺ T cells and B cells.^{125,126} Activation of natural killer (NK) cells expressing KIR3DS1 and KIR3DL1 receptors has been associated with slowing of the HIV disease progression.¹²⁷ Additionally, NK cells can curb viraemia through efficient cytolysis and production of cytokines and chemokines. However, HIV-1 modulates the expression of ligands for NK cell receptors, greatly impairing the capacity of NK cells to kill infected cells.¹²⁸

2.5.3 The T cell response to HIV-1 infection

Following mucosal transmission of HIV, viral RNA is not detectable in the circulation for about 10 days, after which, the plasma RNA levels increase exponentially to over a million RNA copies per mL of blood at 21–28 days after HIV infection.¹¹² The control of viral replication occurs 12-20 weeks after infection as CD8⁺ T cell responses peak.⁴⁶⁻⁴⁸ The earliest CD8⁺ T cell responses target the viral Env and Nef, and are important for controlling acute viraemia, while, the later T cell responses target the conserved Gag p24 and Pol proteins, and are required to maintain the viral load at the set point.¹²⁹⁻¹³¹ The peak in CD8⁺ T cell

responses drive selection of escape mutants resulting in virus diversification as viraemia declines to the set point.^{117,132} After the control of acute viraemia, the number of CD4⁺ T cells is usually restored to normal in the blood but not in the GALT.^{119,122,123}

2.5.4 The antibody response to HIV-1 infection

The initial antibody response to HIV-1 infection is non-neutralizing.^{133,134} HIV-1 gp41 specific antibody responses are the first to appear, arising approximately two weeks after detection of viral RNA in the blood. Generation of antibodies to HIV-1 gp120 is delayed for a further two weeks.¹³⁵⁻¹³⁷ These early anti-gp41 and anti-gp120 antibody responses are ineffective; they neither control initial viraemia nor select escape mutations.¹³⁸ The first antibodies that neutralize autologous virus appear after the initial control of viraemia, 12 weeks or longer after HIV-1 transmission.^{38,39} The delay in generation of these potentially protective antibodies could be attributed to early loss of germinal centres in the gut.¹³⁹

HIV-1 induces massive B cell apoptosis, lysis of follicular B cells, and a loss of up to 50% of germinal centres within the first 80 days of infection.^{137,140} However, these autologous virus-neutralizing antibodies only recognize epitopes bound on the transmitted Env variant.^{38,39} Antibodies that neutralize heterologous viruses are not produced during acute HIV-1 infection, but are seen in 20% of patients 20-30 months after infection.^{39,141,142} These antibodies target conserved regions of HIV-1 Env such as the carbohydrate epitopes, the CD4-binding site, and the membrane-proximal region.¹⁴³⁻¹⁴⁵ The late generation of broadly neutralizing antibodies could be ascribed to delay in affinity maturation caused by impaired CD4⁺ T cell help.¹¹²

3 RESEARCH AIMS

3.1 AIM

The primary goal was to investigate HIV vaccine-induced immune responses elicited in Tanzanian vaccinees primed with HIV-DNA and boosted with HIV-MVA with or without envelope protein (CN54rgp140/GLA-AF) and to explore the suitability of female sex workers for participation in an HIV vaccine efficacy trial.

3.2 SPECIFIC OBJECTIVES

- Study I: To determine the frequency and durability of binding antibodies to the scaffolded V1V2 protein after priming with HIV-DNA and boosting with HIV-MVA vaccines.
- Study II: To determine the impact of giving Env protein (CN54rgp140/GLA-AF) boost together with HIV-MVA immunization on the frequency and breadth of anti-V1V2 responses induced in individuals primed with HIV-DNA vaccine.
- Study III: To assess the impact of vaccine-induced seroreactivity on the performance of HIV diagnostic algorithms in sub-Saharan Africa.
- Study IV: To determine the incidence of HIV in a cohort of high-risk female volunteers for participation in an HIV vaccine efficacy trial.

4 MATERIALS AND METHODS

4.1 STUDY VACCINES

The HIVIS and TaMoVac trials were phase I/II HIV vaccine clinical trials in which participants were immunized with a vaccine regimen consisting of priming with HIV-DNA and boosting with HIV-MVA, either alone or together with CN54rgp140/GLA-AF (**study I, II, and III**). The HIV-DNA is a multigene, multisubtype vaccine composed of seven plasmids encoding HIV-1 gp160 (subtypes A, B, C), p17/p24 gag (subtypes A, B), and rev and RT (subtype B).¹⁴⁶ The HIV-MVA contains a live, non-replicating, genetically modified strain of vaccinia virus that expresses Env of CRF01_AE, and Gag-Pol genes from HIV-1 subtype A.¹⁴⁷ The CN54rgp140 is a clade C recombinant Env protein constructed from a Chinese HIV-1 isolate 97CM001, clone p97CN54,^{148,149} while GLA-AF is an emulsion free adjuvant that targets toll-like receptor 4 (TLR4) on dendritic cells.¹⁵⁰ The HIV-DNA injections were delivered intradermally with a needle free device while HIV-MVA and Env protein immunizations were given intramuscularly.

4.2 STUDY DESIGN

The capability of the HIV-DNA prime HIV-MVA boost vaccination strategy to elicit potent and durable anti-V1V2 Env responses was assessed in healthy, HIV uninfected HIVIS03 volunteers who had agreed to participate in a follow up study three years after completion of the HIVIS03 trial. In the HIVIS03 study, the volunteers were immunized with three HIV-DNA injections given at weeks 0, 4, and 12 and two HIV-MVA boosts administered at months 9 and 21.¹⁵¹ Three years after completion of the HIVIS03 trial, 20 vaccinees were invited to receive a third HIV-MVA boost in the HIVIS06 trial.¹⁵² Samples collected at four weeks post the second HIV-MVA immunization, and three years afterwards, before giving the third HIV-MVA boost, were analysed for induction and persistence of V1V2 antibody responses (**Study I**). The vaccination schedule for study I is summarized in Figure 6.

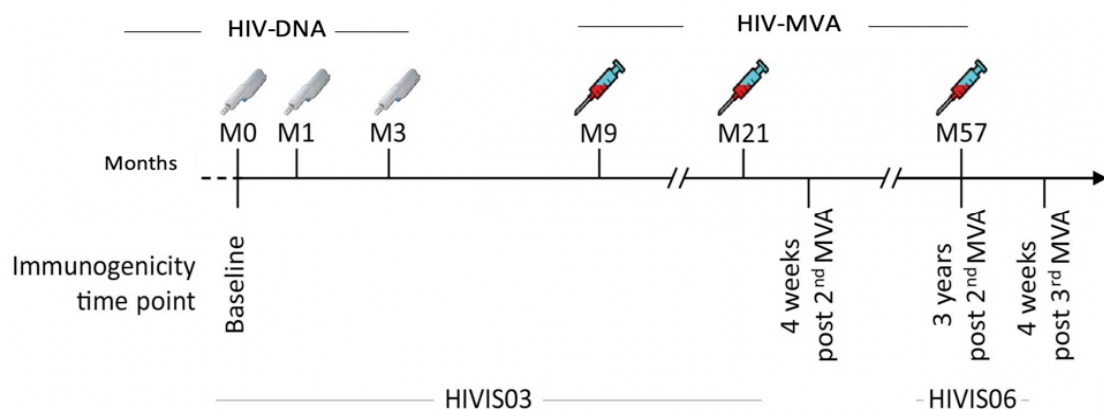


Figure 6. Vaccination schedule and immunogenicity testing time points in the HIVIS03 and HIVIS06 vaccine trials (Study I).

The effect of simultaneous boosting with Env protein and HIV-MVA on induction of anti-V1V2 responses was examined by comparing immune responses elicited in HIV-DNA primed volunteers boosted with both HIV-MVA and CN54rgp140/GLA-AF to those induced in volunteers boosted with HIV-MVA alone (**Study II**). In the TaMoVac II trial, participants were randomized twice; first into three groups to receive 600µg HIV-DNA either as; i) two intradermal (ID) injections only ii) two ID injections with electroporation iii) one ID injection with electroporation. The HIV-DNA immunizations were administered at weeks 0, 4, and 12. Upon completion of priming immunizations, the vaccinees were randomized into two groups to receive two HIV-MVA boosts, either given alone (group 1) or in combination with CN54rgp140/GLA-AF (group 2). The boosting vaccinations were administered 16 weeks apart. Samples collected at baseline and four weeks after the last immunization were used to compare the frequency and magnitude of V1V2 specific responses between the two boosted groups. A summary of the vaccination schedule for study II is provided in Table 3.

Table 3. Vaccination schedule for TaMoVac II participants (Study II)

| First Randomization (Priming immunizations at weeks 0, 4, 12) | | | |
|--|---|--|--|
| Group | A | B | C |
| Immunization* | Two ID HIV-DNA injections (total 600µg) | Two ID HIV-DNA injections (total 600µg) with EP | One ID HIV-DNA injection (total 600µg) with EP |
| Second randomization (Boosting immunizations at weeks 24, 40) | | | |
| Group | 1 | 2 | |
| Immunization | 10 ⁸ pfu HIV-MVA IM | HIV-MVA 10 ⁸ pfu IM plus 100 µg CN54rgp140/GLA-AF | |

* ID; Intradermal. EP; electroporation.

The impact of vaccine induced seroreactivity (VISR) on the accuracy of HIV-RDTs and ensuing performance of HIV testing algorithms was assessed by using 180 samples collected from healthy, HIV uninfected vaccine recipients at the time of peak immune responses. The vaccinees had received three HIV-DNA primes and two HIV-MVA boosts with or without Env protein in previous HIVIS and TaMoVac trials.¹⁵¹⁻¹⁵⁶ The HIV diagnostic algorithms of Tanzania and Mozambique were applied to determine the extent to which VISR will confound diagnosis of HIV among vaccine recipients in sub-Saharan Africa (**Study III**).

PrEPVacc is a phase IIb HIV vaccine trial that evaluates the efficacy of concurrent use of HIV vaccines and pre-exposure prophylaxis (PrEP) in averting HIV infections. Two vaccine regimens; HIV-DNA-Env Protein and HIV-DNA-MVA-Env Protein are being compared to a placebo control, and an oral PrEP regimen; daily TAF/FTC (Descovy) is being compared to daily TDF/FTC (Truvada).¹⁵⁷ A preceding registration cohort comprising of 700 female sex workers (FSW) was established. The FSW were recruited using respondent driven sampling

(RDS) method and followed up every three months to assess their suitability for enrolment into the PrEPVacc trial. The HIV incidence and retention rate observed after 12 months of follow up were analysed to decide whether the FSW in Dar es Salaam are a suitable target population for participation in efficacy trials (**Study IV**).

4.3 IMMUNOGENICITY ASSESSMENT

4.3.1 Induction of anti-V1V2 IgG responses (Study I and II)

Vaccine induced IgG antibodies capable of binding to the gp70 V1V2 protein (CRF01_AE A244, subtype C CN54, subtype C Consensus C, and subtype B Case A2) were detected using enzyme-linked immunosorbent assay (ELISA). Briefly; diluted vaccinees' samples were added to plates that had been coated with recombinant gp70V1V2 protein (Immune Technology Corp., New York, NY, USA) for 18-24 hrs. The V1V2 specific IgG antibodies were detected using sheep antihuman IgG conjugated with horseradish peroxidase (IgG-HRP, Binding Site, Birmingham, UK) and visualized using ABTS peroxidase substrate system (KPL, Gaithersburg, MD, USA).

4.3.2 Assessment of V1V2 IgG subclass responses (Study I and II)

Like for total IgG, V1V2 specific IgG1, IgG2, IgG3, and IgG4 responses were detected using ELISA. Sheep anti-human IgG1-HRP, IgG2-HRP, IgG3-HRP, and IgG4-HRP (Binding Site, Birmingham, UK) were used to detect V1V2 IgG subclasses and ABTS peroxidase substrate system (KPL, Gaithersburg, MD, USA) to visualize the reaction.

4.3.3 Surface plasmon resonance assay (Study I)

IgG Antibody responses recognizing Cyclic V2 peptides (A244 CRF01_AE, subtype C Consensus C and subtype B MN) were measured using Biacore T100.

4.3.4 Depletion of IgG antibodies (Study II)

For detection of Env specific IgA antibodies, IgG was depleted from vaccinees' samples using protein G Sepharose 4 Fast Flow beads (GE Healthcare, Buckinghamshire, UK) and depletion of reactivity confirmed using anti-Env IgG ELISA.

4.3.5 Induction of Env-specific IgA antibodies (Study II)

Stimulation of plasma IgA responses targeting the HIV-1 Env was investigated in ELISA using IgG depleted vaccinees' samples. The samples were added to plates coated with HIV-1 subtype C gp140 and incubated overnight at 4 to 8°C. Following washes, biotinylated goat antihuman IgA (Southern Biotech, Birmingham, AL, USA) was added to detect elicited human IgA antibodies and the reaction visualized using HRP-conjugated streptavidin (Southern Biotech, Birmingham, AL, USA) and OPD peroxidase substrate (SIGMAFAST OPD tablet set).

4.3.6 Measurement of ADCC-mediating antibodies (Study II)

IgG antibodies that mediate ADCC activity were measured in a Luciferase assay using target cells infected with Env.IMC.LucR virus subtype CRF01_AE HIV-CM235-2 LucR.T2A.ecto/293T(IMCCM235) (GenBank accession no. AF259954.1). Effector cells were derived from peripheral blood mononuclear cells of healthy HIV uninfected donors. Polyclonal IgG from HIV infected donors (HIVIG- Division of AIDS, NIAID, NIH) and serum from an HIV seronegative individual were used as positive and negative controls, respectively.

4.3.7 Mapping of IgG responses against the HIV-1 Env (Study II)

Antigenic epitopes recognized by IgG responses to Env immunogens of HIV-MVA and CN54rgp140 vaccines as well as to Env sequences of HIV-1(subtypes A, B, C, CRF01_AE, and CRF02_AG) were mapped using custom-designed linear peptide microarray. Fluorescence intensity (FI) values higher than 2500 after subtraction of baseline reactivity were considered positive and amino acid sequence logos were created based on mean FI responses.

4.4 ASSESSMENT OF VACCINE-INDUCED SEROREACTIVITY (STUDY III)

The ability of HIV diagnostic algorithms to discriminate VISR from true HIV infection was assessed using samples collected from healthy, HIV uninfected volunteers immunized with HIV-DNA-MVA ± Env protein. The samples were tested for VISR using Alere Determine™ HIV-1/2 (Alere Medical Co. Ltd, Japan), Uni-Gold™ HIV-1/2 (Trinity Biotech, Ireland), SD Bioline HIV1/2 (Standard Diagnostic Inc, Republic of Korea), Enzygnost HIV Integral 4 ELISA (Siemens, Germany) and HIV western blot assay (MP Diagnostics, Eschwege, Germany). Thereafter, the HIV testing algorithms of Mozambique and Tanzania were applied.

4.5 HIV, HEPATITIS, SYPHILIS, AND PREGNANCY TESTING (STUDY IV)

HIV testing was performed in line with the Tanzanian HIV testing algorithm which uses two RDTs in series; SD Bioline HIV1/2 (Standard Diagnostics Inc., Republic of Korea) and Uni-Gold HIV-1/2 (Trinity Biotech, Ireland) for screening and confirmation of infection, respectively. HIV was diagnosed when both assays were reactive. Discrepant results were resolved by ELISA. Hepatitis B and C infections were screened using RDTs and positive results were confirmed by respective Murex ELISAs (Diasorin S.P.A., Italy). Syphilis was diagnosed when samples were reactive in both Laborex rapid treponemal assay (Orient Gene Biotech Co Ltd, Zhejiang, China) and treponema pallidum haemagglutination assay (TPHA, Chronolab, Barcelona, Spain). Pregnancy testing was performed using Laborex pregnancy rapid test (Orient Gene Biotech Co Ltd, Zhejiang, China).

4.6 DATA ANALYSIS

GraphPad PRISM, R and STATA were employed to perform statistical analysis of immunological data. Fisher's exact test and Mann–Whitney test were respectively used to compare the frequency and magnitude of antibody responses elicited at the same immunization time point, whereas, McNemar test and Wilcoxon matched-paired signed rank test were respectively used to compare the frequency and levels of antibody responses elicited at two different immunization time points. Logistic and Poisson regressions were applied to determine factors associated with HIV prevalence and to estimate the one-year HIV incidence rate, respectively. A two-tailed p-value < 0.05 was statistically significant.

4.7 ETHICAL CONSIDERATIONS

The HIVIS01/02/05 and HIVIS07 trials received ethical approvals from the Regional Ethics Committees in Stockholm and the Swedish Medical Products Agency. The HIVIS03 and HIVIS06 protocols and products were approved by Tanzania's National Health Research Ethics Committee (NatHREC) of the National Institute of Medical Research, the Senate Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS), the Mbeya Medical Research Ethics Committee, and the Tanzania Food and Drugs Authority (TFDA). The TaMoVac01 and TaMoVac02 clinical trials were approved by Tanzania's NatHREC, the MUHAS Senate Research and Publications Committee, the Mbeya Medical Research Ethics Committee, TFDA, the National Health Bioethics Committee of Mozambique, the Pharmaceutical Department of the Ministry of Health in Mozambique, and the Regional Ethics Committee in Stockholm, Sweden. The PrEPVacc registration cohort was approved by Tanzania's NatHREC and the Senate Research and Publications Committee of MUHAS. During the study period, PrEP was not available at the study site (MUHAS) due to limited availability of the drugs in the country. Back then, the government had not yet endorsed the use of PrEP as a preventive measure against HIV, hence, the drugs were only available at PrEP demonstration projects and research programs. However, at each clinic visit, study participants (FSW) were provided with health education on the effectiveness of PrEP in preventing HIV infections and were encouraged to access the drugs through available channels. Consequently, 8% of the FSW reported PrEP use in the follow up visits.

5 RESULTS

5.1 PAPER I: ANTI-V1V2 IgG RESPONSES INDUCED BY HIV-DNA PRIMING AND HIV-MVA BOOSTING PERSISTED FOR THREE YEARS

The durability of elicited V1V2 antibody responses was monitored in 20 vaccinees who had received three HIV-DNA immunizations at months 0,1,3 and three HIV-MVA boosts at months 9, 21, and 57 (Fig 6). The three-year interval between the second and third HIV-MVA boosts provided an opportunity to examine the persistence of V1V2 antibody responses generated after three HIV-DNA primes and two HIV-MVA boosts. Anti-Env V1V2 (CRF01_AE A244, subtype C CN54, subtype C consensus C, and subtype B Case A2) responses were analysed at three time points; four weeks after the second HIV-MVA immunization, at the time of the third HIV-MVA (three years after the second HIV-MVA), and four weeks after the third HIV-MVA boost.

5.1.1 V1V2 total IgG response

Only 19 vaccinees' samples were available for V1V2 IgG testing four weeks after the second HIV-MVA boost. A sample from one vaccinee could not be tested due to insufficient volume. IgG antibodies binding to the V1V2 Env of CRF01_AE A244 were found in 100% (19/19) of tested vaccinees four weeks after the second HIV-MVA boost and were maintained for three years in 75% (15/20) of the vaccinees. The response rate was 95% (19/20) after the third HIV-MVA immunization, a non-significant increase (Table 4). High levels of anti-CRF01_AE A244 V1V2 IgG responses (median titer 3200 (IQR; 1600–12800)) were elicited four weeks after the second HIV-MVA, but were not sustained for three years, with the median titers dropping significantly to 300 (IQR; 50–800) $p = 0.001$. The third HIV-MVA boost significantly enhanced antibody levels, increasing the median titers to 1600 (IQR; 800–3200) $p < 0.0001$ (Fig 7A).

Four weeks after the second HIV-MVA, anti-V1V2 subtype C CN54 responses were detected in 47% (9/19) of the vaccinees, with median titers of 800 (IQR; 400-2400) among the nine responders. However, the frequency of IgG responses declined considerably to 10% (2/20), $p = 0.016$, three years after the second HIV-MVA, and the third boost did not significantly improve the frequency nor the magnitude of IgG antibodies binding to subtype C CN54 V1V2 region (Table 4, Fig 7B). Anti-V1V2 subtype B Case A2 responses were rare, while, IgG responses to the V1V2 of subtype C Consensus C were not detected (Table 4, Fig 7C).

Table 4. Frequency of V1V2 IgG and IgG subtypes in HIVIS06 trial

| Ab | Antigen gp70V1V2 | Subtypes | Positive/Total Number Tested (%) | | | | |
|--------------|---------------------|----------|---|--|---|--------|--------|
| | | | Four weeks after the 2nd HIV-MVA ^a | At the time of the 3rd HIV- MVA ^b | Four weeks after the 3rd HIV-MVA ^c | a vs b | b vs c |
| Total | A244 | AE | 19/19 (100) | 15/20 (75) | 19/20 (95) | 0.125 | 0.125 |
| IgG | CN54 | C | 9/19(47) | 2/20 (10) | 4/20 (20) | 0.016 | 0.5 |
| | Case A2 | B | 3/19 (16) | 0/20 | 2/20 (10) | 0.25 | 0.5 |
| | Consensus C | C | 0/20 | 0/20 | 0/20 | | |
| IgG1 | A244 | AE | 17/20 (85) | 5/20 (25) | 13/20 (65) | 0.000 | 0.008 |
| IgG2 | A244 | AE | 0/20 | 0/20 | 0/20 | | |
| IgG3 | A244 | AE | 3/20 (15) | 0/20 | 1/20 (5) | 0.25 | 1 |
| IgG4 | A244 | AE | 1/20 (5) | 0/20 | 0/20 | | |
| IgG1 | CN54 | C | 3/9 (33) | 0/9 | 2/9 (22) | 0.25 | 0.5 |
| | Case A2 | B | 0/3 | 0/3 | 0/3 | | |

Anti-V1V2 responses four weeks after the second HIV-MVA immunization (a), three years after the second HIV-MVA (b) and four weeks after the third HIV-MVA boost (c).

Ab: Antibody.

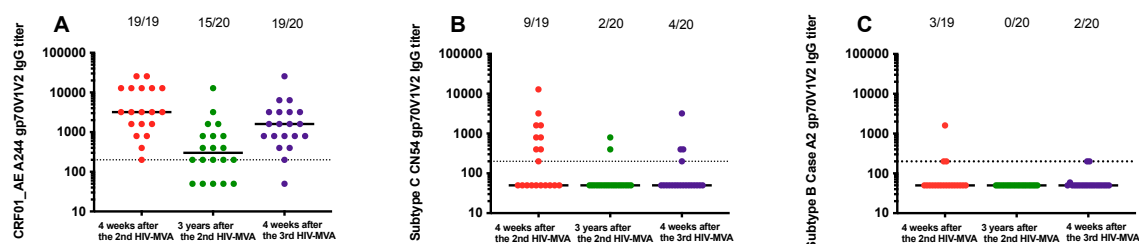


Figure 7. Durability of anti-V1V2 IgG responses

5.1.2 V1V2 IgG subclass response

The anti-CRF01_AE A244 V1V2 IgG response was dominated by the IgG1 subtype, with 85% (17/20) of vaccinees exhibiting CRF01_AE A244 V1V2 specific IgG1 antibodies four weeks after the second HIV-MVA boost. Three years later, at the time of the third HIV-MVA boost, IgG1 responses were still detectable in 25% (5/20) of the vaccine recipients, and the frequency increased significantly after the third HIV-MVA vaccination, rising to 65% (13/20), $p = 0.008$ (Fig 8A). Anti- CRF01_AE A244 V1V2 IgG3 responses were elicited in 15% (3/20) of the vaccinees four weeks after the second HIV-MVA, were undetectable at the time of the third HIV-MVA immunization, and could not be boosted by the third HIV-MVA (Fig 8B). IgG2 antibodies against V1V2 Env of CRF01_AE A244 were not detected, whereas, anti-CRF01_AE A244 V1V2 IgG4 antibodies were found in one individual only (Table 4).

Additionally, there was considerable waning of the magnitude of anti-CRF01_AE V1V2 IgG1 responses over the three-year period, with antibody titers declining from a median of 400 (IQR; 125–800) four weeks after the second HIV-MVA boost to undetectable levels ($p < 0.0001$). The magnitude of anti-CRF01_AE V1V2 IgG1 responses was not significantly increased by the third HIV-MVA boost (Fig 8A).

Anti-V1V2 IgG1 (subtype C CN54 and subtype B Case A2) responses were assessed in samples with V1V2 total IgG reactivity. Subtype C CN54 V1V2 IgG1 specific responses were detected in one-third (3/9) of the responders four weeks after the second HIV-MVA boost. The responses waned with time and were undetectable three years later. The third HIV-MVA vaccination did not boost anti-subtype C CN54 V1V2 IgG1 responses. IgG1 responses to the V1V2 Env of subtype B Case A2 were undetectable (Table 4).

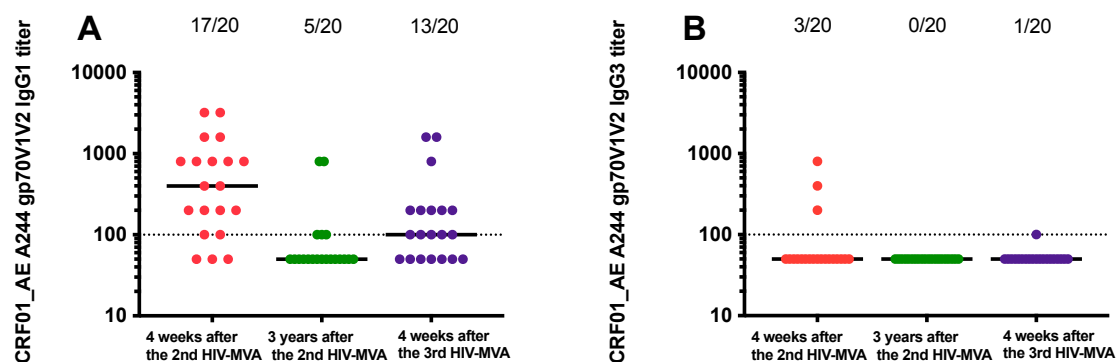


Figure 8. Durability of V1V2 IgG1 and V1V2 IgG3 responses.

5.2 PAPER II: ANTIBODY RESPONSES ELICITED BY HIV-DNA PRIMING AND HIV-MVA BOOSTING WERE NOT AUGMENTED BY ADDITION OF ENV PROTEIN (CN54RGP140/GLA-AF)

HIV-DNA primed volunteers were randomized in two vaccination arms to be boosted with either two HIV-MVA vaccinations alone (group 1) or two HIV-MVA vaccinations co-administered with CN54rgp140/GLA-AF (group 2). Samples collected four weeks after the last immunization were analysed to compare the frequency, breadth, and magnitude of binding antibody responses induced in the two boosting groups.

5.2.1 IgG responses to the scaffolded gp70V1V2 proteins in CRF01_AE and subtype C Env

A high proportion of vaccinees generated IgG antibodies to the scaffolded gp70V1V2 proteins in CRF01_AE and subtype C Env. Binding antibodies to CRF01_AE V1V2 were detected in more than three quarters (77%) of vaccinees, while, nearly two-thirds (65%) had subtype C V1V2 specific responses. The frequency of V1V2 IgG responses induced in the two boosting arms were comparable. Anti-CRF01_AE V1V2 responses were detected in 80% of vaccinees boosted with HIV-MVA alone (group 1) and in 74% of HIV-MVA + CN54rgp140/GLA-AF recipients (group 2), $p = 0.4309$. IgG responses against subtype C gp70V1V2 were elicited in 66% of group 1 and 63% of group 2 vaccinees, $p = 0.8611$.

Similarly, there was no significant difference in the magnitude of the responses. The median titers of IgG responses targeting the V1V2 loop of CRF01_AE were 800 (IQR; 200–1600) and 800 (IQR; 20–1600) in group 1 and 2 vaccinees, respectively, $p = 0.6754$. Anti-subtype C gp70V1V2 titers were 400 (IQR; 20–800) in group 1 and 400 (IQR; 20–1600) in group 2, $p = 0.7384$ (Figure 9A and 9B).

Analysis of IgG1 and IgG3 responses showed a high frequency of anti-CRF01_AE V1V2 IgG1 responses in both immunization arms, with 78% and 76% of vaccinees in group 1 and 2, respectively, exhibiting IgG1 responses. Unlike IgG1, anti-CRF01_AE V1V2 IgG3 responses were substantially more frequent in group 1 (38%) than in group 2 (8%) vaccinees, $p = 0.0132$.

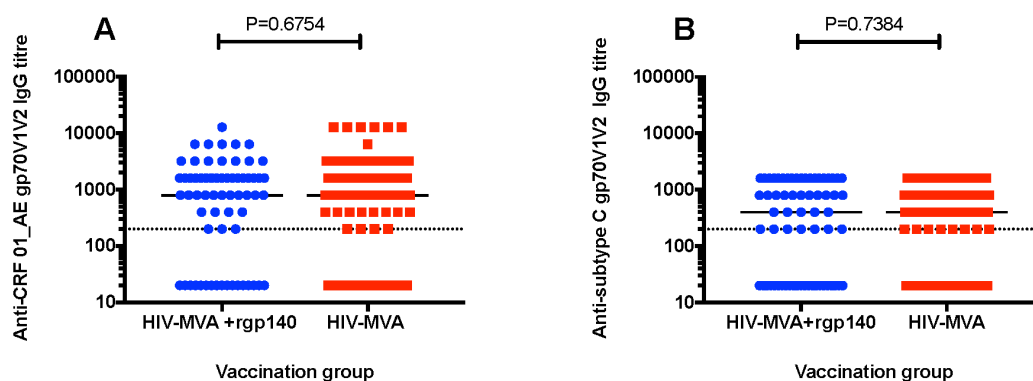


Figure 9. Frequency and magnitude of V1V2 IgG in the two vaccination arms.

5.2.2 IgA antibodies against subtype C Env

To detect IgA in plasma, IgG was depleted from vaccinees' samples. Overall, IgA antibodies were elicited in few vaccinees (18%), however, the response rate was significantly higher in group 2 (vaccinees receiving DNA-MVA-Env protein, 36%) than in group 1 (recipients of DNA-MVA only, 3%), $p = 0.0031$.

5.2.3 ADCC-mediating antibodies against CM235 CRF01_AE

The frequency and magnitude of ADCC responses to CM235 CRF01_AE infected cells were comparable between the two boosting arms. The functional antibody responses were detected in 24% of group 1 and 18% of group 2 vaccinees, $p = 0.5282$. The median titers among responders in group 1 and 2 were 1312 (IQR; 424–2496) and 572 (IQR; 417–939), respectively, $p = 0.125$.

5.2.4 Env antigenic epitopes targeted by vaccine-induced IgG responses

The elicited IgG responses targeted five immunodominant regions in the HIV-1 Env; V1V2, C2, V3, C4, and gp41. Both boosting vaccine regimens induced IgG antibodies against the V1V2, V3, and gp41, however, the C2 and C4 specific responses were detected in group 2 only (Table 5).

Analysis of the antibody recognition pattern in the V1V2 region revealed that, IgG responses in both boosting arms recognized the same peptide variants in the V2 loop. The frequency of responses was comparable between the two groups, though the intensity was slightly higher in group 1 than in group 2. Preferential targeting of amino acids E¹⁶⁴, K¹⁶⁹, and VH¹⁷²⁻¹⁷³ was noted. The sequence of recognized amino acids matched the protein sequence in HIV-MVA.

IgG antibodies that target the V3 region were elicited in most of the vaccinees, however, the responses were substantially stronger and broader in group 2 vaccinees, $p < 0.0001$. Amino acids S³⁰⁶, I³⁰⁹, and FY³¹⁵⁻³¹⁶ were preferentially targeted, and sequencing revealed high homology with vaccine inserts sequences. A mismatch was observed with clade B HIV-DNA only.

Antibodies recognizing the C2 and C4 regions were elicited by the group 2 regimen (HIV-MVA+ CN54rgp140/GLA-AF) only. The targeted amino acid sequences were highly similar to the CN54 rpg140 sequence. Anti-C4 antibodies showed a preferred recognition of amino acids K⁴⁴⁰ and Q⁴⁴²/N⁴⁴².

The frequency and intensity of IgG responses targeting gp41 peptides were comparable in the two groups. The sequence similarity between recognized amino acid and vaccine immunogens was high and elicited antibodies had a preference for amino acids V⁵⁸³ and K/R⁵⁸⁸.

Table 5. Immunodominant Env regions recognized by vaccine-induced IgG responses

| IDR | Peptide Position | HXB2 Position | Env Region | Representative Sequence | FOR (%) | | | mean FI | | |
|--------|------------------|---------------|------------|-------------------------|----------|------------|---------|----------|------------|---------|
| | | | | | MVA only | MVA+rgp140 | Placebo | MVA only | MVA+rgp140 | Placebo |
| 1_V2 | 176 | 164 | V2 | ELRDKKQKVHALFYK | 68 | 63 | 0 | 20,587 | 14,486 | 0 |
| 2_C2 | 221 | 200 | C2 | AITQACPKVTFDPIP | 25 | 72 | 9 | 0 | 9,188 | 0 |
| 3_V3 | 321 | 300 | V3 | GNNTRKSIRIGPGQT | 21 | 84 | 9 | 0 | 18,855 | 0 |
| | 322 | 301 | V3 | NNTRKSIRIGPGQTF | 64 | 91 | 18 | 23,543 | 40,829 | 0 |
| | 325 | 304 | V3 | RKSIRIGPGSTFYAT | 68 | 100 | 18 | 25,586 | 45,585 | 0 |
| | 326 | 305 | V3 | KSVRIGPGQTFYATG | 82 | 97 | 27 | 28,859 | 43,633 | 12,656 |
| 4_C4 | 461 | 433 | C4 | AMYAPPIAGNITCKS | 25 | 75 | 27 | 0 | 16,693 | 9,640 |
| 5_gp41 | 612 | 580 | gp41 | VLAVERYLKDQKFLG | 86 | 78 | 0 | 11,773 | 13,694 | 0 |

5.3 PAPER III: A CONSIDERABLE PROPORTION OF HIV VACCINEES WILL BE MISCLASSIFIED BY HIV DIAGNOSTIC ALGORITHMS IN SUB-SAHARAN AFRICA

Many studies have investigated the frequency of VISR among participants in HIV vaccine clinical trials, but not its impact on the performance of HIV testing algorithms. Here, we report how detection of vaccine-induced antibodies in HIV negative volunteers will potentially affect the accuracy of RDTs in diagnosis of HIV.

5.3.1 HIV testing algorithms misdiagnosed a substantial proportion of HIV vaccinees

Vaccinees' samples collected four weeks after the last vaccination were tested for reactivity in HIV RDTs, ELISA, and Western blots. When HIV diagnostic algorithms were applied; 26.3% of vaccinees would have been incorrectly regarded as HIV positive in Mozambique,

where serial testing with Alere Determine™ HIV-1/2 and Uni-Gold™ HIV-1/2 is employed. The misclassification would have been twice as high (54 %) in Tanzania, where HIV infection is diagnosed by sequential testing with SD Bioline HIV-1/2 3.0 and Uni-Gold™ HIV-1/2. Nevertheless, the highest misclassification (93.4%) occurred when Enzygnost HIV Integral 4 ELISA was used. Additionally, between 22% and 99% of vaccine recipients would have received false positive results if HIV testing was performed using Western blot assay. HIV misdiagnosis was influenced by the level of induced antibody responses. The median titers of subtype C Env-specific antibodies were significantly higher among misdiagnosed vaccinees than in those with HIV negative results. However, reactivity in RDTs decreased over time, and three years after completion of the HIV-DNA-MVA immunizations, none of the vaccinees would have received a wrong HIV diagnosis, neither in Mozambique nor in Tanzania.

5.4 PAPER IV: PREVALENCE AND INCIDENCE OF HIV AMONG FEMALE SEX WORKERS BEING CONSIDERED FOR PARTICIPATION IN A PHASE IIB HIV VACCINE EFFICACY TRIAL

A total of 700 FSW enrolled into a registration cohort were followed up for one year to determine their suitability for participation in the PrEPVacc trial. HIV prevalence, incidence, associated factors, and retention rate were reported at the end of the follow up period.

5.4.1 Prevalence of HIV and other sexually transmitted infections

At the time of enrolment, FSW were screened for HIV, syphilis, and hepatitis B (HBV) and C virus (HCV) infections. The baseline prevalence was 7.6% (95% CI of 5.8%-9.7%) for HIV, 1.2% (95% CI 0.5%-2.2%) for syphilis, 1.7% (95% CI 0.9%-2.6%) for HBV, and 1.0% (95% CI 0.5%-2.0%) for HCV infection. The likelihood of being HIV positive at enrolment was higher among older FSW (>24 years), illicit drug users and those with sexually transmitted infections (STIs).

5.4.2 HIV incidence in the cohort

After one year of follow up, the 700 participants contributed 609 person years at risk (PYR) to the study. Attendance was good with a retention rate of 80%. During follow up, 21 FSW seroconverted, generating an incidence of 3.45 per 100 PYR (95% CI: 2.25-5.29/100 PYR). Although analysis of risk factors for infection was limited by the low number of seroconversions, we noted that the HIV incidence rate was high among FSW who were; 1) young, 18-24 years 2) separated/divorced/widowed 3) using illicit drugs 4) pregnant at enrolment 5) who had completed secondary education 6) who did not use condoms the last time they had sex, and 7) who had STIs at baseline.

6 DISCUSSION

6.1 VACCINE-INDUCED ANTIBODY RESPONSES TO THE HIV-1 V1V2 ENV REGION

The binding of IgG to the gp70V1V2 protein was identified as a primary correlate of reduced risk of HIV-1 infection in the RV144 vaccine efficacy trial.^{58,60,158} As shown in study II, the HIV-DNA/MVA regimen elicited robust IgG responses to the V1V2 Env of HIV-1 CRF01_AE in more than three quarters (77%) of healthy African vaccinees, and to subtype C V1V2 in nearly two-thirds (65%) of the same vaccinees. The binding of the V2 domain of gp120 to $\alpha_4\beta_7$ integrin plays an important role in establishment of HIV infection after mucosal transmission. Through this interaction, the V2 loop mediates activation and proliferation of CD4⁺ T cells, thereby increasing their susceptibility to HIV. Moreover, it facilitates the trafficking of $\alpha_4\beta_7$ -expressing CD4⁺ T cells to the gut-associated lymphoid tissues where viral seeding occurs.¹⁵⁹⁻¹⁶² A group of $\alpha_4\beta_7$ blocking antibodies have been isolated from vaccinees in the RV144 trial and after late boosting of a subset of RV144 vaccinees in the RV305 trial using AIDSVAX B/E with or without ALVAC-HIV.¹⁶³ Although the $\alpha_4\beta_7$ blocking does not prevent HIV infection, induction of anti-V2 antibodies capable of inhibiting Env- $\alpha_4\beta_7$ binding may decrease HIV infection risk by preventing viral adhesion to highly susceptible cells.^{159,160,164}

As reported in study II, priming with three HIV-DNA vaccinations and boosting with two HIV-MVA vaccinations elicited IgG antibodies that recognized linear epitopes in the V1V2, V3, and gp41 Env regions. The V2-specific responses preferentially targeted peptides with sequences closely matching the HIV-MVA. Positivity rates were highest for amino acids E¹⁶⁴, K¹⁶⁹, and VH¹⁷²⁻¹⁷³. Analysis of breakthrough infections in the RV144 trial showed that vaccine-induced V1V2 responses were capable of blocking infections by viruses that matched the vaccine strains at position K¹⁶⁹ of the V1V2 loop.¹⁶⁵ Furthermore, V2-specific monoclonal antibodies isolated from RV144 vaccinees identified position K¹⁶⁹ (lysine) as an antigenic target for protective immune responses.¹⁶⁶ We found that co-administration of CN54 rgp140/GLA-AF with the HIV-MVA boost did not improve the V2 response. Immunogen structure and sequence have been reported to impact IgG responses induced by vaccination.¹⁶⁷ Great sequence differences existed between the CN54 rgp140 and the recognized V2 peptides, and this could have contributed to the failure of Env protein boost to enhance anti-V2 responses.

The importance of sequence homology in optimizing V1V2 responses was also illustrated in two phase I/IIa canarypox prime-protein boost trials conducted in South Africa. The trials aimed at; 1) determining whether protective immune responses seen in the RV144 trial would be elicited in an African population immunized with the RV144 regimen (HVTN097),¹⁶⁸ and 2) evaluating the immunogenicity of the RV144 protocol when adapted to subtype C (HVTN100).¹⁶⁹ The HVTN100 regimen elicited higher levels of Env-specific antibodies than the RV144 regimen. However, the frequency and magnitude of V1V2 responses induced by the subtype C regimen were substantially lower than those elicited by the RV144 regimen,

with positivity rates of 61% and 100% respectively.¹⁷⁰ Additionally, the IgG responses elicited in HVTN100 preferentially recognized V2 sequences of AE.A244 over those of subtype C, indicating that envelope sequences expressed by HIV-1 strains in priming and boosting immunogens impact elicitation of anti-V1V2 responses.¹⁷⁰

The HVTN100 regimen was designed to improve the efficacy of the RV144 regimen.¹⁶⁹ After exceeding the prespecified threshold for efficacy testing, the HVTN100 regimen consisting of priming with ALVAC-HIV vector (vCP2438) and boosting with MF59-adjuvanted bivalent subtype C gp120 was evaluated for efficacy in the region where HIV-1 subtype C is prevalent.¹⁷¹ Unfortunately, the regimen failed to prevent HIV infections. Differences in vaccine strains, adjuvants, dosage, interval between boosting immunizations, study population, and genetic diversity of circulating strains likely contributed to the lack of efficacy in the HVTN702 trial.¹⁷¹ These disappointing results call for revisiting of basic science research to improve HIV immunogen designs and vaccine concepts.¹⁷²

In the RV144 trial, antibody responses that correlated with low HIV infection rates declined soon after vaccination. The rapid decline in V1V2 specific responses was in parallel with the waning of vaccine efficacy from 60.5% at 12 months post-initial vaccination to 31.2% at 42 months.⁶² Thus, increased durability of anti-V1V2 responses is important for vaccine efficacy.^{173,174} As reported in study I, the HIV prime-boost DNA-MVA regimen induced long-lasting V1V2 IgG responses in a majority of vaccine recipients. At peak immunogenicity, 97% of vaccinees had IgG antibodies against the V1V2 loop of CRF01_AE A244 and the responses were sustained for three years in 75% of them, though at substantially lower titers. A late HIV-MVA boost significantly increased the antibody titers. Additionally, delayed boosting with HIV-MVA restored IgG recognition of the same linear Env antigenic epitopes targeted at peak immunogenicity.¹⁷⁵ Increased durability of IgG responses that contributed to the partial vaccine efficacy was observed in RV144 vaccinees given additional AIDSVAX B/E boost either alone or in combination with ALVAC-HIV, 9-12 months after completion of primary vaccination series.¹⁷⁶ However, boosting after 6-8 years failed to increase durability of anti-V1V2 responses despite improving the titers.¹⁷⁷ Evaluation of the impact of vaccine type on durability of elicited antibody responses showed that MVA-boosted HIV regimens generate Env-specific antibodies with longer half-lives but lower peak magnitudes than protein-boosted vaccinations.¹⁷⁸

RV144 vaccinees with high levels of anti-V1V2 IgG1 and IgG3 responses were found to be at low risk of acquiring HIV.^{62,179} In study I, IgG1 dominated the V1V2 response in HIV-DNA primed HIV-MVA boosted vaccinees. More than three quarters had binding IgG1 antibodies against the V1V2 domain of CRF01_AE A244 V1V2, while just over a third had V1V2-specific IgG3 responses to the same strain. Three years after peak immunogenicity, anti-CRF01_AE A244 V1V2 IgG1 responses were still maintained in a quarter of vaccinees, while anti-V1V2 IgG3 were undetectable. The durability of V1V2 IgG3 responses was also short lived in the RV144 trial.⁶² Although both V1V2 specific IgG1 and IgG3 responses mediated antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular

phagocytosis (ADCP) in protected RV144 vaccinees,¹⁷⁹ IgG1 has been shown to be the main driver of antibody effector functions.¹⁸⁰ In the absence of IgG3 responses, functional activities were maintained in RV144 vaccinees with high IgG1 levels. However, when IgG1 was depleted, vaccinees with high IgG3 responses exhibited significantly lower levels of functional activities. This means that IgG3 alone is insufficient to mediate robust effector functions, pointing to a likely synergy between vaccine-induced IgG1 and IgG3 antibodies to enhance polyfunctionality.¹⁸⁰

In study II, Env-specific IgA responses were more frequent among vaccinees boosted with a combination of HIV-MVA and Env protein compared to those who received HIV-MVA boost only. Induction of high levels of plasma IgA was associated with decreasing vaccine efficacy due to IgA blocking of IgG mediated ADCC.⁶¹ However, profiling of humoral responses in RV144 vaccinees suggests a duo role for IgA.¹⁸⁰ The depletion of IgA from plasma reduced complement activity (ADCD), neutrophil phagocytosis (ADNP) and natural killer (NK) cells degranulation, implying that IgA augments these biological activities. However, the loss of IgA increased levels of IFN- γ and MIP-1 β secretion, indicating impediment of secretion of these cytokines by IgA.¹⁸⁰

An efficacious HIV vaccine must provide universal protection against diverse HIV-1 strains.³⁰ In study II, although the HIV-DNA/MVA regimen elicited potent and durable V1V2 responses, the breadth of the induced responses was narrow. Most of the IgG responses were directed against the V1V2 Env of subtypes AE and C, while targeting of the subtype B V1V2 domain was rare. This difference could have stemmed from Env immunogen sequences in priming and boosting immunizations. The HIV-DNA plasmids encoded HIV-1 Env (subtypes A, B, C) while the HIV-MVA expressed Env of CRF01_AE.¹⁴⁷ Broadening of the sequence diversity will increase the breadth of humoral and cellular responses elicited by the HIV-DNA/MVA.

6.2 VACCINE-INDUCED SEROREACTIVITY IMPACTS DIAGNOSIS OF HIV IN SUB-SAHARAN AFRICA

The majority of healthy, HIV uninfected volunteers participating in HIV clinical trials develop antibodies to HIV after vaccination.¹⁸¹ The detection of these antibodies by standard HIV serological tests is referred to as vaccine-induced seroreactivity (VISR).¹⁸¹ We found that VISR will significantly impact HIV diagnosis in low-income countries that primarily rely on rapid diagnostic tests (RDTs) for routine HIV testing. More than half (54%) of vaccinees immunized with HIV-DNA-MVA \pm Env protein would have been wrongly identified as HIV infected in Tanzania, where serial testing with SD Bioline HIV-1/2 3·0 and Uni-Gold™ HIV-1/2 is used for diagnosis of HIV infection. Similarly, a considerable proportion (26·3%) of the same vaccinees would have been misclassified as HIV positive in Mozambique where sequential testing with Alere Determine™ HIV-1/2 and Uni-Gold™ HIV-1/2 is used for HIV diagnosis. Many countries in sub-Saharan Africa employ either Uni-Gold™ HIV-1/2, Alere Determine™ HIV-1/2, or SD Bioline HIV-1/2 3·0 in their HIV diagnostic strategies (Table 2). As new, more complex, HIV vaccine regimens continue being evaluated for efficacy in

sub-Saharan Africa, the frequency and persistence of VISR will increase, further confounding the diagnosis of HIV in sub-Saharan Africa.¹⁸¹ Taking into consideration the emotional and social trauma inflicted by HIV misdiagnosis on trial participants and their families, it is imperative to develop point of care HIV diagnostic tests that are able to differentiate VISR from true HIV infection.^{72,182}

6.3 SUITABILITY OF FEMALE SEX WORKERS FOR ENROLMENT IN HIV VACCINE EFFICACY TRIALS

The FSW in Dar es Salaam are suitable for enrolment in HIV vaccine efficacy trials. They have a high HIV incidence (3.45/100 PYR, 95% CI 2.25-5.29) and can be recruited and retained in prospective cohort studies. The HIV incidence was higher than that reported from studies of FSW in Nairobi (2.2 %, 95 % CI 1.6–3.1 %),¹⁸³ and Cape Town (2.7/100 PYR, 95% CI: 1.7-4.2).¹⁸⁴ The lower than expected HIV incidence rates in the Nairobi and Cape Town cohorts were mainly attributed to high attrition rate (42%) and wrong recruitment strategy, respectively.^{183,184} Findings from a modeling study suggested that FSW participating in HIV prevention trials in settings with high HIV incidence do not have a similar risk of HIV acquisition. A substantial proportion of them have a low exposure to HIV,¹⁸⁵ underscoring the importance of a good recruitment strategy.

Recruiting a cohort with consistently high exposure to HIV is important for an accurate estimate of the efficacy of a vaccine regimen.⁸⁸ The high HIV incidence among Dar es Salaam FSW was similar to that observed in a randomized open label trial assessing the risk of HIV infection among women using different hormonal contraceptive methods in four African countries; Kenya, Eswatini, South Africa, and Zambia.¹⁸⁶ The high HIV infection rate among young FSW (18-24 years) reflects the general vulnerability of young women to HIV infection and the need to increase participation of this group in HIV prevention trials. In March 2022, more than half (56%) of participants enrolled in the PrEPVacc trial at the MUHAS site were less than 25 years old.

The high retention rate (80%) reaffirms the suitability of FSW in our cohort for participation in large efficacy trials. A comparable one-year retention rate was also reported from a study conducted to assess acceptability of HIV vaccine research among FSW in Uganda.⁸⁷ Every effort should be made to minimize attrition since loss to follow-up may lead to underestimation of the HIV incidence if participants who drop out have higher risk behaviours than those who remain.^{187,188} In the PrEPvacc registration cohort, the majority of volunteers who did not complete the follow up visits (n=138) were younger, reported inconsistent condom use and had multiple sexual partners. The high dropout rate among younger FSW in HIV prevention trials has been reported across east Africa,^{87,183,187} signifying the need to devise strategies that improve the research experience of young FSW in HIV clinical trials. Apart from affecting the incidence, early attrition of higher risk participants will also diminish the effectiveness of risk reduction counselling in HIV vaccine efficacy trials. At the MUHAS site, to increase cohort retention, participants are reminded of their clinic visits by phone calls, and we also ask volunteers to assist in tracing their peers who

miss clinic visits. The tracers must be living or working in the same catchment area as lost-to-follow-up participants. Furthermore, we regularly update contact and locator information.

6.4 WHY WAS IT POSSIBLE TO DEVELOP SARS-COV-2 VACCINES BUT NOT HIV-1 VACCINES?

A licensed vaccine against HIV-1 is still missing 38 years after the virus was identified as the causative agent of AIDS. However, it took less than a year since the start of the coronavirus disease 2019 (COVID-19) pandemic to develop several effective vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).^{189,190} Immunological and virological differences between SARS-CoV-2 and HIV-1 are behind the rapid development of COVID-19 vaccines.

Unlike in HIV-1, most people infected with SARS-CoV-2 recover and acquire immunity.¹⁹¹ HIV-1 infected individuals do not clear the virus (except in rare cases) since it integrates into the host genome within three days of transmission, establishing an irreversible infection.¹⁸⁹ Analysis of SARS-CoV-2 specific immune responses in individuals who had recovered from COVID-19 made it easier to design, evaluate and deploy anti-SARS-CoV-2 vaccines within a very short period of time.¹⁹⁰

Effectiveness of vaccines against most viral diseases depend on their ability to elicit broadly neutralizing antibodies against their targets. The ease by which SARS-CoV-2 neutralizing antibodies are induced after vaccination contributed to the rapid development of COVID-19 vaccines. While anti-SARS-CoV-2 neutralizing antibodies develop within two weeks of vaccination, to date, it has not been possible to elicit antibodies capable of neutralizing HIV-1 by vaccination.^{141,189} Nonetheless, HIV-1 broadly neutralizing antibodies develop in a few individuals (10-30%) two to four years after infection.^{141,192-194}

The high mutation rate of HIV-1 and the resultant mutational escape are key hurdles to the development of an effective HIV-1 vaccine.^{95,96} In contrast, SARS-CoV-2 is a slowly mutating virus with limited ability to escape host immune responses. The low genetic diversity of SARS-CoV-2 allowed COVID-19 vaccines based on wild-type strain to provide global protection despite emergence of several variants.¹⁹⁵

Knowledge acquired from research on HIV -1 vaccine platforms and delivery techniques informed the rapid development of COVID-19 vaccines.¹⁹⁶ Chimpanzee adenovirus^{197,198} and a rare human adenovirus¹⁹⁹ evaluated as vectors for antigenic sequences in the HIV vaccine field were used as viral vectors in COVID-19 vaccines (ChAdOx-1 nCoV-19 and Ad26.COV2.S).^{200,201} Prime-boost strategies frequently used in HIV vaccine research were employed in COVID-19 vaccinations to improve the strength and diversity of vaccine-induced immune responses.¹⁹⁰ Moreover, sites and programmes used for HIV efficacy trials were mobilized for rapid efficacy testing of COVID-19 vaccine candidates.

7 CONCLUSIONS

The HIV-DNA/MVA vaccination strategy is capable of inducing antibody responses associated with decreasing the risk of HIV infection. The induced V1V2 IgG responses showed remarkable durability in the majority of vaccinees and could be restored by a single HIV-MVA boost after a three-year interval, suggesting that the HIV-DNA/MVA regimen induces long lasting memory responses.

Addition of Env protein (CN54rgp140/GLA-AF) boost does not enhance anti-V1V2 responses elicited by the HIV-DNA/MVA regimen, but generates a broader V3 response and increases Env regions recognized by elicited IgG responses. This signifies the importance of matching immunogen sequence with circulating viral sequences to obtain optimal protection.

The accuracy of rapid test-based HIV diagnostic algorithms in sub-Saharan Africa is substantially compromised by vaccine-induced HIV antibodies, putting healthy HIV negative vaccine recipients at risk of being misclassified as HIV positive. There is an urgent need to develop HIV-RDTs capable of distinguishing VISR from true HIV infection.

The FSW in Dar es Salaam should be considered for enrolment in future HIV vaccine efficacy trials. The high HIV incidence and retention rates observed in the PrEPVacc preparatory study make them suitable for participation in a longitudinal HIV vaccine study.

8 POINTS OF PERSPECTIVE

The good immunogenicity of the HIV-DNA HIV-MVA prime boost vaccine concept, and the excellent durability of the induced V1V2 IgG responses have paved the way for the HIV-MVA and CN54rgp140 vaccines to be included in one of the vaccination arms in the ongoing PrEPVacc HIV vaccine efficacy trial in Tanzania, Uganda, and South Africa.

Since HIV-1 subtype C dominates the epidemic in southern Africa, and contributes significantly to the epidemic in East Africa there is a need to improve the potency and durability of induced subtype C-specific immune responses by increasing the coverage of subtype C strains in the immunogens.

Increased efforts are needed to avert false positive HIV diagnoses in the areas where HIV-1 vaccine efficacy trials have been conducted. Health care providers and policy makers in sub-Saharan Africa should be made aware of the potential impact of VISR on the accuracy of HIV diagnostic algorithms. Given the devastating consequences of HIV misdiagnosis and of failure to detect true HIV infection, a differential HIV-RDT will be needed for use in low-income countries before an effective HIV-1 vaccine receives approval for mass immunization.

The high HIV incidence among young FSW calls for more efforts to increase their recruitment and retention in HIV vaccine efficacy trials, as well as in other HIV prevention activities. Strategies should be tailored to empower the young FSW to overcome social and community barriers that hinder their participation in HIV prevention trials. A combination of biomedical, behavioural, and structural interventions will be required.

The availability of PrEP has implications for future HIV-1 vaccine efficacy trials. Since the consistent use of PrEP is highly effective in decreasing the risk of HIV-1 acquisition, trial designers are ethically required to provide PrEP to all participants as part of a standard prevention package. The high efficacy of PrEP will decrease the HIV incidence in the trial which is needed for statistical power of the study. As a result, demonstration of vaccine efficacy will necessitate expanding the sample size or increasing the follow up periods, substantially impacting the cost of conducting HIV prevention trials.

Identifying immunogens that can elicit antibodies capable of neutralizing HIV-1 remains a major challenge in the HIV vaccine field. Experimental medicine vaccine trials (EMVTs) are being proposed to accelerate discovery of these immunogens. In the EMVTs, several immunogens are evaluated and the ones with a higher probability of success in eliciting broadly neutralizing antibodies are advanced to clinical testing. This will reduce the number of unsuccessful HIV-1 vaccine efficacy trials and increase the likelihood of developing an efficacious HIV-1 vaccine.

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