GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 04 | Issue: 03

On 15th June 2018

http://www.research.lk

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GARI Publisher | Public Health-HIV | Volume: 04 | Issue: 03

Article ID: IN/GARI/ICHM/2018/114 | Pages: 150-166 (17)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 18.05.2018 | Publish: 15.06.2018

Author: - De Silva. U, Kotalawala. S
BMS School of Science, Sri Lanka | GARI Publisher | Public Health-HIV | Volume: 04 | Issue: 03
Article ID: IN/GARI/ICHM/2018/114 | Pages: 150-166 (17)
ISSN 2424-6492 | ISBN 978-955-7153-00-1
Edit: GARI Editorial Team | Received: 18.05.2018 | Publish: 15.06.2018

AN EVALUATION OF STRATEGIES IN PHASE IIB/III TRIALS FOR HIV-1 VACCINATION OF AT-RISK COMMUNITIES

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ABSTRACT

The acquired immunodeficiency syndrome (HIV/AIDS) pandemic continues to endanger human lives with a current prevalence of 36.7 million. The clinical significance of HIV-1 vaccination is as a form of prophylaxis to control incidence. A safe and effective HIV-1 vaccine has been a subject of extensive research surpassing three decades during which a multitude of candidate vaccines were investigated in clinical trials including six efficacy trials. The common objective of the trials has been the immunisation of at-risk communities by vaccination. A preventative HIV-1 vaccine has proved to be elusive as the RV144 trial remains the only trial to have reported a partial efficacy (31.2%) thus far. The immune correlates of the RV144 trial underpin the possibility and the utility of an effective HIV-1 vaccine in spite of prior inefficacious vaccines. The cause of the inefficacies has been identified as an insufficiency of magnitude and diversity in the vaccine-induced immune responses. The review evaluates the advancement of strategies to eliminate the limitations associated with HIV-1 vaccination in relation to efficacy trials. Keywords: HIV-1 vaccination, efficacy trials, vaccination strategies, immune correlates

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INTRODUCTION

The human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) has persisted as a health burden for more than three decades, with 36.7 million people living with HIV (PLHIV) and 1.8 million new infections in 2016. The countries mainly affected by the epidemic are in sub-Saharan Africa (Figure 1). The widespread use of antiretroviral therapy (ART) and prevention modalities such as circumcision programmes and practice of safe sex, have caused a significant decline in the global incidence over the past two decades (United Nations Children's Fund [UNICEF], 2018; Fettig et al., 2014).

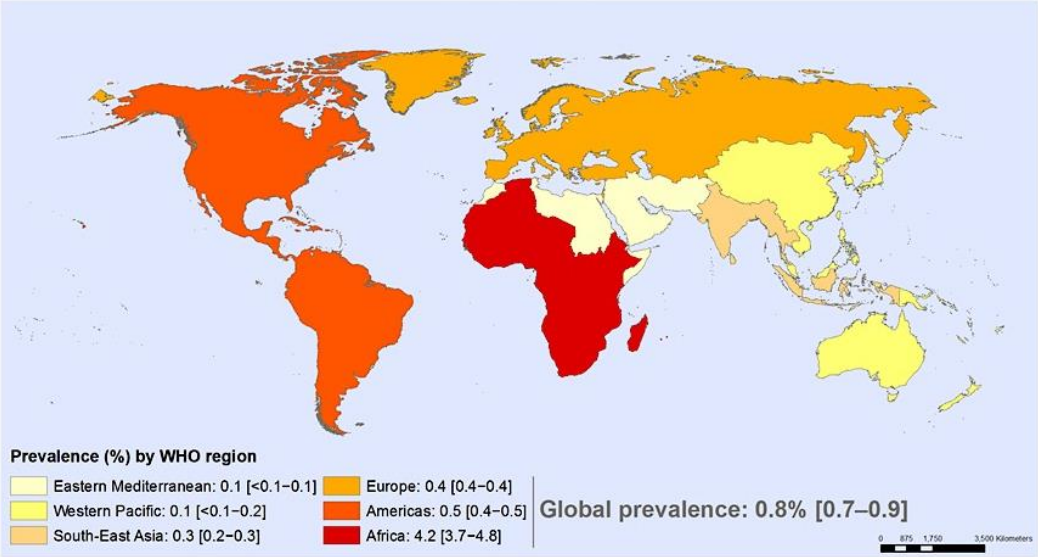

Figure 1. The global prevalence of HIV/AIDS (World Health Organization [WHO], 2018)

A preventative HIV-1 vaccine is regarded the most effective method of disease control, as it provides a safe and cost-effective alternative to ART and prevention strategies whose limitations include inaccessibility and lack of adherence (Jongwe et al., 2016).

The Joint United Nations Program on HIV/AIDS (UNAIDS) created a three-part global target in 2013 known as the 90-90-90 target, to increase diagnosis, treatment and viral suppression. The combination of vaccination with prevention modalities may possibly decrease HIV acquisition by the exertion of a synergistic effect, bolstering the necessity for a HIV vaccine (Medlock et al., 2017).

The main challenge in HIV vaccine development is the induction of an immune response sufficiently effective and diverse to kill virions of any HIV-1 strain before viral latency (Mann and Ndung'u, 2015).

The prospect of a HIV-1 vaccine has been an important subject of research since 1986 in spite of little success. Nevertheless, one candidate vaccine has produced a considerable efficacy proving that developing a vaccine is not impossible (Esparza, 2013). The gp160 vaccine was the first of a multitude of HIV-1 candidate vaccines investigated in clinical trials. These clinical trials include six phase IIb/III trials constituting different candidate vaccines, strategies and schemes (Table 1) (Fuchs et al., 2010).

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Table 1. The efficacy testing of HIV-1 vaccination strategies and vaccine candidates

Trial	Phase	Strategy	Vaccines	HIV incidence per 100 person-years	Result	Reference
Vax 004	III	Monomeric HIV-1 gp120 subunit vaccine	AIDSVAX B/B'®	Overall: 2.6% Men: 2.7% Women: 0.8%	No efficacy	Flynn et al., 2005
Vax 003	III	Monomeric HIV-1 gp120 subunit vaccine	AIDSVAX B/E®	3.4%	No efficacy	Pitisuttithum et al., 2006
HVTN 502	IIb	Ad5-based vaccine	MRKAd5 HIV-1 gag/pol/nef vaccine	Vaccine (Men): 4.00% Placebo (Men): 2.12% Vaccine (Women): 0.00%	No efficacy	Buchbinder et al., 2008

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				Placebo (Women): 0.66%		
HVTN 503	IIb	Ad5-based vaccine	MRKAd5 HIV-1 gag/pol/nef vaccine	Vaccine: 4.54 Placebo: 3.70	No efficacy	Gray et al., 2011
RV 144	III	Prime boost	ALVAC-HIV gp120 AIDSVAX B/E	Vaccine: 0.192 Placebo: 0.279	31.2% efficacy at 42 months	Rerks-Ngarm et al., 2009
HVTN 505	IIb	Prime boost	DNA vaccine rAd5 vaccine	Vaccine: 0.018 Placebo: 0.014	No efficacy	Hammer et al., 2013

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The strategies implemented in HIV vaccine efficacy trials

Monomeric HIV-1 gp120 subunit vaccines

The HIV-1 envelope glycoprotein (Env) is a trimer comprising of the gp160 precursor, surface gp120 glycoprotein and the transmembrane gp41 glycoprotein (Pincus et al., 2017). The Env antigen of the HIV-1 has demonstrated a pivotal role in efficacy trials due to the induction of robust antibody-mediated immune responses, and is the major neutralisation antigen of the HIV-1 (Karasavvas et al., 2015; Khattar et al., 2013).

The Env glycoprotein facilitates viral infection of the host cells, and is the only viral antigen expressed on the surfaces of virions and virally infected cells. The viral adaptation of Env variation among the vast number of HIV-1 strains is known to be problematic in vaccine development (Witt et al., 2017).

Vax004

The bivalent AIDSVAX B/B' vaccine contains 300 μg each of the MN and GNE8 recombinant gp120 HIV-1 Env epitopes from two subtype B strains in 600 μg of alum adjuvant. Flynn et al. investigated the efficacy of the AIDSVAX B/B' in a phase III trial including a study population of 5108 men who have sex with men (MSM) and 309 women at a risk of heterosexual HIV-1 acquisition from North America and the Netherlands. The vaccine and placebo were administered to the respective cohorts at months 0, 1, 6, 12, 18, 24 and 30 via the intramuscular route (Flynn et al., 2005).

The AIDSVAX B/B' had a general tolerance with most adverse events (AEs) being mild or moderate during the first three days following a vaccination. A higher occurrence of symptoms at the injection site such as local oedema and induration, was reported in the vaccine cohort. There were no other significant differences in the number and type of AEs reported between the vaccine and placebo groups (Flynn et al., 2005).

The vaccine was designed to elicit neutralising antibodies (NAbs) against the HIV. The vaccinated subjects developed antibodies against the HIV-1 with higher peak levels of MN CD4-blocking, GNE8 CD4-blocking or MN-neutralising responses, corresponding with a lower rate of infection (Balasubramanian et al., 2018; Flynn et al., 2005). The subgroup analyses of the Vax004 trial indicated an efficacy in female and non-white vaccine recipients. A study by Balasubramanian et al. investigating the antibody responses to the V1V2 and V3 epitopes in the VaxGen trials, indicated that antibody responses targeting the V2 and V3 viral epitopes were more prolonged among female vaccinees (Balasubramanian et al., 2018). A longitudinal analysis of the Vax004 trial indicated that antibody responses targeting the V2 epitope peaked following two or three vaccinations but did not heighten with subsequent doses (Karnasuta et al., 2017). The majority of the vaccinated subjects demonstrated moderate to high titers of NAbs to the MN antigen. The tier 1 viruses represent strains demonstrating a high susceptibility to neutralisation, whereas the tier 2 viral strains are capable of concealing epitopes causing a low susceptibility to neutralisation. A study by Gilbert et al. identified low NAb responses against tier 2 viruses being consistent with the lack of protection in the Vax004 trial (Gilbert et al., 2010).

The vaccine efficacy (VE) of the AIDSVAX B/B' was defined as (1 – the relative risk of infection) × 100 in the Vax004 trial. There was no observable decrease in HIV-1 acquisition among the vaccinated subjects (VE 6% [95% CI -17% to 24%], $p = 0.59$). The cause of the inefficacy of the AIDSVAX B/B' is likely to be due to the lack of NAbs targeting genetically varying primary HIV-1 isolates (Flynn et al., 2005).

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Vax003

The Vax003 trial was conducted in Thailand to assess the bivalent AIDSVAX B/E containing 300 μg each of the MN and A244 rgp120 HIV-1 Env antigens adsorbed onto 600 μg of alum adjuvant. The epitopes were from a CXCR4-dependent laboratory-adapted subtype B strain (MN) and a CCR5-dependent primary subtype CRF01_AE isolate (A244). The epidemic of HIV-1 initiated in Thailand during the late 1980s when the HIV-1 subtype B transmitted rapidly among intravenous drug users (IDUs) and the subtype E (CRF01_AE) transmitted by sexual transmission. Pitisuttithum et al. studied the efficacy of the AIDSVAX B/E vaccine among IDUs randomising the population to a 1:1 vaccine: placebo ratio. The vaccine and placebo were administered at months 0, 1, 6, 12, 18, 24 and 36 via intramuscular injection (Pitisuttithum et al., 2006).

The vaccine produced a moderate safety with tenderness at the site of injection (71.0% vaccine cohort, 65.7% placebo cohort) being the most common AE with no aggravation with subsequent injections. There were no significant differences between the vaccine and placebo cohorts in terms of deaths (Pitisuttithum et al., 2006).

Gottardo et al. analysed the Env peptide binding antibodies to evaluate the significance of non-NAbs binding linear peptides in the Vax004, Vax003 and RV144 trial. The assessment of Env-specific plasma IgG indicated the C5a component of the C5 region of gp120 as a dominant linear B-lymphocyte epitope for the IgG response (Gottardo et al., 2013).

The Table 2 shows the characterisation of the 211 infections in the test population of 2527 subjects, who were HIV-negative at enrolment.

Table 2. The characterisation of the 211 infections used to assess VE (adapted from Pitisuttithum et al., 2006)

Parameter	Vaccine (n=106)	Placebo (n=105)
Infections with were subtype CRF01_AE	83	81
Infections with were subtype B′	14	18
Infections with were subtype B	1	0
Infections with untypeable subtype/s	14	

The VE of the AIDVAX B/E was determined as (1 – relative risk of infection) × 100%, and was estimated to be 0.1% ([95% CI -30.8% to 23.8%], p = 0.99). The study concluded that the vaccine failed to protect against HIV-1 acquisition or delay disease progression with no efficacy towards either subtype B or/and subtype E. The cause of the inefficacy has been attributed to that of the Vax004 trial (Pitisuttithum et al., 2006).

Vector-based vaccines

The data of the VaxGen trials bolster that vaccine-induced immune responses do not necessarily confer protection, as the immune responses required for protection against infection or control of viraemia have not been substantiated (Hopkins et al., 2014). Antibodies, $CD8^+$ cytotoxic T-lymphocytes (CTLs), $CD4^+$ T-lymphocytes and HIV-specific memory B-lymphocytes are crucial components of vaccine-induced control of HIV infections (Moyo et al., 2017; Hopkins et al., 2014; Priddy et al., 2008).

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Vector-based vaccines represent a promising approach for vaccine design by which modified viruses expressing HIV-specific genes, infect a cell population culminating in the intracellular expression of the HIV genes as transgenes (Gu et al., 2013).

The human adenovirus family constitutes over 70 serotypes of non-enveloped DNA viruses. The extensive use of adenoviruses as vaccine vectors is based on their high packing capacity, high immunogenicity, safety and ability to infect dividing and non-dividing cells (Kiener et al., 2018).

Replication-deficient recombinant adenovirus type 5 (rAd5)-based vaccines have been studied extensively as the vector is among the most immunogenic for cellular immunity vaccines (Hopkins et al., 2014; Hertz et al., 2013). The adenovirus vector is capable of expressing a high amount of antigen and polyvalent mosaic antigens. These antigens are generally expressed as transgenes, but may otherwise be expressed as exogenous peptides. The strategy referred to as 'antigen capsid-incorporation', is to induce a potent antibody response due to the expression of antigens on the adenoviral capsid (Gu et al., 2013).

The gag (*gag* gene from HIV-1 strain CAM-1), pol (*pol* gene from HIV-1 strain IIIB) and nef (*nef* gene from HIV-1 strain JR-FL) epitopes are intracellular HIV-1 proteins, and are effective in activating $CD8^+$ T-lymphocytes for immunisation (Hopkins et al., 2014).

The trivalent MRKAd5 HIV-1 gag/pol/nef vaccine is a 1:1:1 formulation of three recombinant subtype B Ad5 vectors expressing the *gag*, *pol* and *nef* genes. The gag, pol and nef epitopes have shown to induce robust CTL responses. Studies using primate models have revealed that Ad5-based vector vaccines expressing HIV-1 *gag*, induce robust CTL responses (Hopkins et al., 2014; Priddy et al., 2008).

HVTN 502

The HVTN 502 trial was designed as a phase IIb study to investigate the MRKAd5 vaccine in regions where the subtype B is the most prevalent. The study by Buchbinder et al. enrolled 3000 HIV-1-seronegative subjects at a high risk of HIV-1 acquisition from 34 locations across North America, the Caribbean, South America and Australia (Buchbinder et al., 2008). A phase I clinical study of the vaccine concluded that it was tolerated and immunogenic among HIV-seronegative adults and Ad5-seropositive subjects (Priddy et al., 2008). The trial was initially envisioned to enrol subjects with low (≤200) Ad5 antibody levels, but was later expanded for subjects with >200 Ad5 antibody levels. The study population was randomised 1:1 (vaccine:placebo) to receive three 1.0 mL intramuscular injections (day 1, week 4, week 26) of either the MRKAd5 vaccine containing $1{\cdot}5\times10^{10}$ adenoviral genomes or the placebo of vaccine diluent (Buchbinder et al., 2008).

The AEs from the vaccine were similar to prior research with pain at the injection site and headache being mostly observed (Buchbinder et al., 2008).

The interim analysis in September 2007 indicated no significant decrease in HIV infections and early viral load in the Ad5 antibody ≤ 200 cohort. The vaccinations were halted, and the study was unblinded due to results meeting pre-specified futility criteria (Duerr et al., 2012; Rolland et al., 2011).

The low rates of HIV transmission precluded a conclusive evaluation of VE among female cohorts (Table 1). A striking observation was the increased incidence in the male cohorts who were uncircumcised and/or had pre-existing Ad5 antibody titers (Buchbinder et al., 2008). The presence of Ad5-specific $CD4^+$ T-lymphocytes has been the proposed explanation for the enhanced HIV-1 infections in Ad5-seropositive subjects (Tenbusch et al., 2012). A

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significant depletion in $CD4^+$ T-lymphocytes occurs during an acute HIV infection impairing the antiviral response. The question of whether HIV-specific $CD4^+$ T-lymphocytes will enhance viral replication is debateable, and the formation of $CD8^+$ T-lymphocytes has been the key strategy in vaccination (Soghoian et al., 2012). A case-cohort analysis by McElrath et al. determined that the response rate and magnitude of $CD8^+$ T-lymphocytes in the HVTN 502 trial had been the largest hitherto. The probable causes of inefficacy in the trial were that the T-lymphocytes were not broadly reactive, and that the single effect of T-lymphocytes is insufficient (McElrath et al., 2008).

An extended follow-up study determined the vaccine:placebo hazard ratio (HR) to be 1.40 ([95% CI 1.03 to 1.92], p = 0.03) among all subjects, and confirmed the vaccine-enhanced risk of infection observed in the interim analysis (Moodie et al., 2015).

HVTN 503

This phase IIb trial was the second study to assess the MRKAd5 vaccine. It was conducted in South Africa, where subtype C is the most prevalent, and included sexually active (largely heterosexual) adults. The study population was randomised 1:1 into vaccine:placebo groups to receive three 1.0 mL intramuscular injections (months 0, 1, 6) of either $1{\cdot}5\times10^{10}$ adenoviral genomes/mL MRKAd5 vaccine or the placebo of vaccine diluent (Gray et al., 2011).

The enrolment and vaccination of subjects was halted in September 2007 due to new developments in the parallel HVTN 502 trial indicating futility (Hopkins et al., 2014).

The vaccine was tolerated with reactogenicity reported from both vaccine and placebo groups – local pain or tenderness, systemic symptoms, headache, fever or fatigue, myalgia, arthralgia, nausea, chills, diarrhoea and vomiting (Gray et al., 2011).

The results of the HVTN 503 trial were in accord with those of the HVTN 502 trial in concluding that the MRKAd5 vaccine was ineffective in preventing HIV-1 acquisition or reducing early viral load in Ad5-seropostive and Ad5-seronegative vaccine cohorts (Gray et al., 2011). A long-term follow up analysis at a median of 42 months by Gray et al. indicated a higher rate of HIV-1 incidence in the vaccine group, and supported the vaccine-enhanced risk of transmission in the male vaccinees observed in the HVTN 502 trial (Gray et al., 2014). A VE was not evident. The HR for infections for male to female cohorts was 1.25 (95% CI 0·76 to 2·05). However, the assessment of VE is limited due to the termination of enrolment and vaccination (Gray et al., 2011).

The $CD4^+$ T-lymphocytes constitute an integral component of the host immunity by initiating signals to other components of the immune system. An antigen-specific immune response requires activation and proliferation of $CD4^+$ T-lymphocytes during a natural infection and immunisation. However, the $CD4^+$ T-lymphocytes are also the major lymphocyte subset targeted by the HIV-1 during infection. Novel research has indicated a variation in susceptibility to infection of $CD4^+$ T-lymphocytes based on the antigens (Auclair et al., 2018). Hu et al. determined that Ad5-specific $CD4^+$ T-lymphocytes are highly susceptible and diminished in HIV-infected individuals during a natural infection and rAd5 vaccination (Hu et al., 2014). The mechanisms by which rAd5-based vaccination enhanced HIV-1 infection in the HVTN 502 and HVTN 503 trials are complex and multifactorial, and bolster the use of alternate vectors in vaccine research (Auclair et al., 2018).

Prime boost

The prime boost concept is the use of a viral vector prime and a soluble Env subunit boost in a single regime to establish immunity by means of antibodies and T-lymphocytes to identify,

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neutralise and kill multiple HIV-1 strains (Pitisuttithum et al., 2011).

RV144

The RV144 trial was a phase III study utilising the combination of a replication-deficient recombinant canarypox prime – ALVAC-HIV [vCP1521] from Sanofi Pasteur (USA) – and a protein boost – gp120 AIDSVAX B/E from Global Solutions for Infectious Diseases (USA) – in the world's first community-based efficacy trial to experiment the prime boost strategy (Chapman et al., 2017; Karasavvas et al., 2015; Pitisuttithum et al., 2011).

The circulating recombinant form (CRF) CRF01_AE and subtype B are the predominant subtypes causing the Thai epidemic of HIV-1 infections (Pitisuttithum et al., 2011). The canarypox vector of the ALVAC-HIV was genetically engineered to express Env gp120 of the CRF01_AE 92TH023 strain linked to the transmembrane component of subtype B gp41 (includes a deletion in the immunodominant region lacking the entire gp41 ectodomain) and the Gag and protease of LAI strain. The ALVAC-HIV was grown in chicken embryo fibroblasts, and was formulated with 10 mM Tris HCl, pH 9 and lactoglutamate as a lyophilised vaccine for injection. The AIDSVAX B/E was a highly purified mixture of the MNgD and A244gD gp120 proteins. The gp120 HIV-1 subtype B MN and HIV-1 gp120 CRF01_AE A244 antigens were each fused with a 27 amino acid sequence from the herpes simplex virus gD protein at their N-termini. These antigens were expressed in Chinese hamster ovary (CHO) cells, adsorbed onto aluminium hydroxide gel adjuvant, and formulated into the vaccine (Karasavvas et al., 2015; Pitisuttithum et al., 2011).

The Thai Ministry of Public Health has recognised the threat of HIV infection, persistently supporting HIV vaccine research in Thailand. The study enrolled 16402 HIV-seronegative subjects aged between 18 and 30 years with behavioural risks of HIV-1 acquisition such as needle sharing and unprotected intercourse (Pitisuttithum et al., 2011; Rerks-Ngarm et al., 2009).

The ALVAC-HIV prime was administered in doses of $10^{6.5}$ $CCID_{50}$ at day 0, week 4 (pre-specified range 3 – 7), week 12 (10 – 15) and week 24 (21 – 28) with boosting (300 μg of each antigen in the AIDSVAX B/E vaccine) performed with the last two prime vaccinations (Karasavvas et al., 2015; Rerks-Ngarm et al., 2009). The placebo group received a sterile, lyophilised preparation of virus stabiliser and 1 mL of 0.4% sodium chloride, and a 600 μg preparation of aluminium hydroxide gel adjuvant instead of the ALVAC-HIV and AIDSVAX B/E vaccines respectively. The ALVAC-HIV vaccine or placebo was administered in the left deltoid muscle, and the AIDSVAX B/E or placebo was administered in the right deltoid muscle (Pitisuttithum et al., 2011).

The results of the study showed that the AEs were similar to those observed in other research investigating the vaccines. The reactogenicity ranged from mild to moderate, and the majority of the reactions resolved three days following an injection (Rerks-Ngarm et al., 2009).

The objective of the prime boost regime is to induce $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocytes, binding antibodies and neutralising antibodies to identify, neutralise and kill multiple strains of the HIV-1 before an infection is established irreversibly (Pitisuttithum et al., 2011). The results of the RV144 trial indicated that a variety of immune responses were organised against the HIV-1 including T-cell-line adapted neutralising antibody, antibody-directed, cell-mediated cytotoxicity, $CD4^+$ lymphoproliferation and $CD8^+$ T-lymphocytes although these responses may not be relevant. The VE of this prime boost regimen was determined to be 31.2% ([95% CI 1.10 to 52.1], $p = 0.04$) at 42 months following

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vaccination. A 60.5% estimated efficacy was reported 6 months following vaccination indicating an early effect of the vaccine that diminished over time (Yates et al., 2014; Rerks-Ngarm et al., 2009).

Haynes et al. highlighted two correlates with infection among the vaccine group – an inverse correlation between IgG antibody binding to scaffolded V1V2 Env, and a direct correlation between IgA antibody binding to Env and Env C1 region. These findings may enable improvements in vaccine design. A vaccine inducing high levels of V1V2 antibodies and low levels of Env-specific IgA antibodies may amplify vaccine efficacy, if the protective properties of V1V2 IgG antibodies can be confirmed (Haynes et al., 2012).

The antibody subclasses – IgG1 to IgG4 – possess variable affinities for Fc receptors giving them different functional attributes in spite of identical epitope specificity. IgG3 antibodies have the longest and most flexible hinge region of IgG subclasses, and have been greatly associated with complement fixing and high affinity for the FcγRI, FcγRII, FcγRIIa and FcγRIII receptors. Yates et al. studied the immune responses of the RV144 trial and Vax003 trial – the latter investigated the single effect of the AIDSVAX B/E vaccine – to demonstrate the significance of Env IgG3 responses. The protein vaccine of the Vax003 trial reportedly generated a higher level of NAbs than the prime boost regime of the RV144 trial. It was determined that the RV144 trial had a higher frequency of IgG3 responses while the Vax003 regime induced higher IgG1, IgG2 and IgG4 responses. The antiviral property of Env IgG3 antibodies was significantly correlated to antibody-dependent cellular cytotoxicity (ADCC) in the RV144 trial but not in the Vax003 trial based on the Wilcoxon rank-based test. It was also determined that the V1V2 IgG3 response rate is significantly higher in the RV144 trial, as it was this response that correlated inversely with HIV-1 infection (Yates et al., 2014). The significant correlation between IgG3 titers with Tier-1 neutralisation and ADCC may have caused the augmented efficacy in the RV144 trial, although it is inconclusive as the two trials used different routes of HIV-1 transmission. The elevated anti-gp70 V1V2 IgG3 responses in the RV144 trial suggested that priming with the ALVAC-HIV may have induced class-switching via T-lymphocyte cytokines and activation of a different subset of responder B-lymphocytes (Karnasuta et al., 2017; Yates et al., 2014).

A study by Zolla-Pazner analysing the V2 antibody responses in the RV144 trial, showed that the V2 antibodies were induced mainly by the subtype E A244 than the subtype B MN gp120 in spite of similar antigenicity. The V2 domain of the A244 rgp120 is more immunogenic, and generally requires three immunisations to elicit seroconversion to the V2 domain. The V2 region of the vCP1521 vector carrying the CRF01_AE *env* gene is almost identical to that of the A244 gp120 but is highly different to that of the MN gp120, leading to the deduction that the antibody response to the CRF01_AE V2 was primed more effectively. ADCC, antibody-dependent cell-mediated virus inhibition, virolysis, opsonisation and virus aggregation are among the likely anti-viral functions of anti-V2 antibodies as the V2 epitope is expressed on the surfaces of virions and infected cells (Zolla-Pazner et al., 2013).

Combinatorial polyfunctionality analysis of antigen-specific T-cell subsets (COMPASS) studies have also indicated the protective effect of cytotoxic polyfunctional $CD4^+$ T-lymphocyte subsets in the RV 144 trial. The moderate efficacy in the RV 144 trial and the results of follow-up analyses have underpinned the strategy of eliciting a combination of humoral and cellular responses (Karnasuta et al., 2017; de Souza et al., 2012).

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Edit: GARI Editorial Team | Received: 18.05.2018 | Publish: 15.06.2018

HVTN 505

The HVTN 502 trial was conducted as a phase IIb study of a DNA/rAd5 prime boost regimen comprising of DNA primes and a trivalent rAd5 vector boost. The study enrolled 2504 fully circumcised, Ad5-seronegative (Ad5 serum NAb titer below 1:18) MSM and transgender women from 21 locations in the USA (Fong et al., 2018; Hammer et al., 2013). The DNA vaccine was a 1:1:1:1:1:1 formulation of Env constructs (for surface gp120 and partial transmembrane gp41) of subtypes A, B and C and Gag, Pol and Nef constructs of subtype B. The rAd5 boost encoded Env of subtypes A, B and C and a Gag-Pol fusion protein of subtype B (deCamp et al., 2017). Plasmid DNA vaccines have demonstrated potential for gene-based antigen delivery. The lack of pre-existing vector immunity, the feasibility of manufacture and the stability of DNA vaccines are advantageous over microbial vector vaccines (Graham et al., 2013). The DNA prime was injected into the deltoid in 4 mg doses at weeks 0, 4 and 8 using a Biojector. The Biojector is a needle-free device shown to enhance humoral responses during DNA vaccine delivery in animal models and human studies. A dose of 10^{10} particle units of rAd5 vaccine was administered into the deltoid at week 24 using needle and syringe. The placebo groups received phosphate-buffered saline and vector-free diluent instead of DNA prime and rAd5 boost respectively (Graham et al., 2013; Hammer et al., 2013).

The regimen produced an acceptable safety profile. The vaccine group had a significant occurrence of reactogenicity in regard to the placebo group with most symptoms ranging from mild to moderate. The HVTN 505 study was halted early as recommended by the data and safety monitoring board due to lack of efficacy. The study's primary analysis indicated a VE of -25.0% ([95% CI -121.2% to 29.3%], p = 0.44). The formation of cellular and antibody lymphocytes was elicited but did not decrease HIV-1 infections or viral-load set point among vaccinees (Hammer et al., 2013).

The role of $CD4^+$ T-lymphocytes in infection may undercut vaccine-induced immunity, and may even be harmful for vaccinated subjects. A study by Hu et al. proposed a number of mechanisms by which Ad5-based vaccination may enhance HIV-1 acquisition including a Th17-like proinflammatory phenotype, a release of proinflammatory cytokines following Ad5 stimulation and an elevated expression of mucosal homing molecules by Ad5-specific $CD4^+$ T-lymphocytes (e.g. $\alpha4\beta7$ integrin, CCL-20). The higher frequency of Ad5-specific $CD4^+$ T-lymphocytes detected in the rectum and colon of vaccinees in the HVTN 505 trial are in accord with the mechanism of Ad5-specific $CD4^+$ T-lymphocytes homing mucosal surfaces (Hu et al., 2014).

A significant inverse correlation between month 7 Env-specific $CD8^+$ T-lymphocytes and risk of infection was noted. The humoral responses demonstrated a poor response to the V1V2 loop but robust IgG responses targeting the gp41. The robust gp41 response impeding any protective responses, and the antibody responses correlating with infection risk have been postulated to be causes of inefficacy. The IgG V1V2 response has been correlated with reduced HIV-1 acquisition in the RV144 trial. The IgG V1V2 response was considerably low in the HVTN 505 trial. However, a study by Fong et al. indicated a decreased risk of infection in vaccine recipients with detectable V1V2 IgG responses (Fong et al., 2018). The strongest correlation of infection risk was demonstrated by Env IgG responses together with Env-specific $CD8^+$ T-lymphocytes, indicating that combining multiple immune responses can confer protection against HIV-1 acquisition, and bolstering the necessity of cellular and humoral immune responses (Fong et al., 2018).

CONCLUSION

HIV-1 vaccination remains a subject of extensive research in human studies and non-human primate models. The common strategy unanimously bolstered by the efficacy trials is the induction of humoral (bNAbs) and cellular responses ($CD8^+$ T-lymphocytes) in vaccinees (Ondondo et al., 2016).

Antibodies constituted a pivotal role in the RV144 trial in spite of an observable futility of bnAbs (Bruel et al., 2017). Passive immunisation against the simian immunodeficiency virus in macaque models has demonstrated prophylactic effects, indicating pre-existent immunity as a means of preventing HIV-1 infection (Euler et al., 2011). Monoclonal antibodies are capable of neutralising a range of HIV-1 strains and clearing the persistent HIV-1 reservoir due to their Fc effector functions – the latter characteristic renders them therapeutically superior to ART (Bar et al., 2016). The VRC01-class of bnAbs is a promising approach in vaccine design offering potency, protective capacity and saturation of in vitro neutralisation curves at 100% viral neutralisation (Jardine et al., 2016).

The broad diversity of the HIV is attributed to the virus' accelerated mutagenesis forming subtypes based on differences in amino acids (Ndhlovu et al., 2011). This phenomenon is particularly important in developing vaccines based on CD8+ T-lymphocytes. The lack of diverse immunogens in the MRKAd5 vaccine is a possible cause of inefficacy in the HVTN 502 trial (Uchtenhagen et al., 2014). Polyvalent mosaic antigens are full-length proteins formed by *in silico* recombination using natural sequences with optimisation by bioinformatics. The technique preserves natural expression and processing of antigens while enabling maximum coverage of HIV-1 strains and $CD8^+$ T-lymphocyte epitopes (Barouch et al., 2013; Barouch et al., 2010).

The HVTN 702 trial is a phase IIb/III, which commenced in South Africa in October 2016, marking the most recent venture into efficacy trials (de Montigny et al., 2018). South Africa has been affected greatly by the HIV/AIDS pandemic, having the highest population of PLHIV (Cornell et al., 2017). The HVTN 100 trial was a phase I-II trial, which investigated a subtype C ALVAC-HIV vaccine and bivalent subtype C gp120/MF59 vaccine in low-risk South African adults. The success of the trial led to the establishment of the HVTN 702 trial with 5400 subjects. It follows the strategy of the RV144 trial, and aims to target the HIV-1 subtype C, which predominates in South Africa. The vaccination scheme will follow a five-dose regime, and will use the ALVAC-HIV vaccine from Sanofi Pasteur and a bivalent subtype C gp120 protein vaccine with MF59 adjuvant from GlaxoSmithKline. The results of the clinical trial are anticipated in 2020 (de Montigny et al., 2018; Ajbani, 2016; Dyer, 2016).

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