

## Vaccines against HIV

Usha Padmanabhan<sup>1</sup>, Abhay S Chowdhary<sup>2\*</sup>, Ritwik Dahake<sup>2</sup> and Prasad S Koka<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Haffkine Institute for Training, Research & testing, India

<sup>2</sup>Department of Virology and Immunology, Haffkine Institute for Training, Research & testing, India

Submission: October 08 2015; Published: October 19, 2015

\*Corresponding author: Abhay S Chowdhary, Department of Virology and Immunology, Haffkine Institute for Training, Research & Testing, Acharya Dhonde Marg, Parel, Mumbai 400012, India, Tel: +91-022-241-60947; Fax: +91-022-24161787; Email: abhaychowdhary@yahoo.com

### Abstract

The human immunodeficiency virus-1 (HIV-1) infects helper CD4(+) T cells, and causes CD4(+) T-cell depletion and immunodeficiency. Although significant progress has been made in antiretroviral therapy in the past 30 years, an effective vaccine and a functional cure awaits discovery. This review summarizes the molecular basis of HIV infection and the various hurdles for vaccine development and in this perspective analyzes the status of vaccines against HIV.

**Keywords:** HIV; Immunology; DNA vaccine; CTL responses

**Abbreviations:** DC: Dendritic Cells; CTL: Cytotoxic T Lymphocytes; TGF: Transforming Growth Factor; VRC: Vaccine Research Center; ELISPOT: Enzyme-Linked Immunosorbent Spot.

### Introduction

#### Molecular basis of HIV infection

The genome of HIV-1 is small, less than 10 kb, encoding nine HIV-1 genes which have been characterized for their products in great detail. Among these the envelope protein called gp160 synthesized as gp120 and gp40 is most well characterized. Since HIV is an encompassed virus, the viral envelope must fuse with the host cell membrane to deliver the viral RNA into the host cell. HIV infects a host cell by attaching to specific receptors on the host via gp120.

#### Pathology of HIV Infection

HIV infection can be divided into 4 stages: (a) Primary Infection (b) Clinically asymptomatic stage (c) Symptomatic HIV infection and (d) Progression from HIV to AIDS.

During primary infection, 20% patients suffer from flu-like symptoms and then the reaction subsides. The body begins to make anti HIV antibodies in a process called sero-conversion at which stage diagnostic antibody tests can be falsely negative because of low levels of serum antibody. During the clinically asymptomatic stage (which lasts for an average of 10 yrs), the virus is active at the lymph nodes, but patients show very little pathological symptoms. HIV antibody tests however are positive. In the symptomatic HIV stage, the patient displays 2 major symptoms: a fall in CD4+ cell and platelet count. The immune system starts to deteriorate and patient is vulnerable to

opportunistic infections. In the final stage, the CD4+ count has dropped to very low levels and patient succumbs to opportunistic infections such as pneumonia, tuberculosis and is usually fatal. How does HIV cause CD4+ T cell depletion? Initially HIV was thought to directly infect CD4+ T cells and causes apoptosis in them. However, the long asymptomatic phase belies this paradigm. It is now thought that HIV causes CD4+ T cell depletion through indirect mechanisms [1].

To understand the mechanisms by which HIV causes CD4+ T cell depletion, let us consider how CD4+ cells usually mount an immune response. Naïve CD4 T cells are activated by an interaction with dendritic cells (DC) that present an antigen. These activated T-cells then rapidly proliferate and differentiate into several subsets of effectors T-cells [2]. While the majority of effectors cells rapidly die, a small minority will survive and undergo a transition to a resting state as memory CD4+ T cells which form adaptive memory or immunogenic memory. Upon future encounter with the same antigen, the memory CD4+ T cells provide for an enhanced immune response and are likely derived from all effectors subsets [3].

Many reports suggest that during the asymptomatic phase which lasts for 10 yrs HIV infects these effectors cells latently by viral genome integration into the mammalian genome where it is transcriptionally inactive and does not produce active virions or by maintaining a very low level of transcription where some but not all viral genes are transcribed without the production of active virions.

## Strategies and difficulties in HIV vaccine development

Based on the mechanism of HIV infection and subsequent progression to disease, HIV vaccine development faces severe challenges. Firstly, most vaccines for other infectious diseases or viruses do not prevent the infection but prevent the disease associated with the infection by mitigating the physiological responses evoked by the infectious agent. The long latent phase of HIV however precludes this approach.

Vaccines that are first generation vaccines however deliver either inactivated viruses or live but attenuated viruses which mimic the immune response of the virulent strain and elicit an immune response from the host that confers immunity to the virulent strain. Using first generation vaccines from inactivated HIV preparations has not been attempted for the fear that the virus will not be completely inactivated, in particular when the HIV virions tend to aggregate. Furthermore HIV is highly mutable. On facing pressure from immune responses in the host, HIV virus typically evolves so that it can escape both the humoral and the cellular immune responses. Part of this escape mechanism is the variation in the antigenic region (epitopes) of envelope proteins and also due to masking of the antigenic region of envelope proteins such as gp120 through glycosylation, changes in conformation and trimerization making it difficult to neutralize through antibodies and leads to ineffectiveness in second generation HIV vaccines.

Second generation vaccines includes peptides (generally encoding an immuno-reactive region of the virus or a cytokine) which are directly injected into the host and also do the job of raising antibodies against the virulent strain. This approach also is prone to failure in case of HIV since HIV isolates belong to several clades and subtypes with a wide genetic variety within an infected population. HIV vaccines based on the viral proteins gp120 and gp160 have been attempted.

The first HIV vaccine developed in the early 90's included recombinant forms of the HIV-1 glycoproteins gp120 and gp160 produced in Chinese hamster ovary cells (i.e. a peptide vaccine designed to provoke humoral immunity). Chimpanzees immunized with the gp120 but not the gp160 peptide was protected against a HIV-1 challenge [4]. These results led to phase I, II and III clinical trials in humans.

These studies revealed that although the vaccine was safe and elicited anti-HIV antibody production, it did not provide significant protection against clinical HIV strains [5,6]. Importantly these studies also revealed that the antibody response was transient and had a half life of 40-60 days [7].

Since then, at least 13 different gp120 and gp160 envelope candidates have been evaluated, in the US predominantly through the AIDs Vaccine Evaluation group. Overall, they have been safe and immunogenic in diverse populations; have induced neutralizing antibody in nearly 100% recipients, but rarely induced CD8+ cytotoxic T lymphocytes (CTL), but not providing significant protection against HIV. Given that HIV targets CD4+ T cells which constitutes part of the cellular immune response, the above observations provoked an intense debate whether the

primary immune responses elicited by the HIV vaccines should be humoral or cellular.

The observations that CD8+ T cells were observed soon after HIV infection in humans [8] and plasma viremia increased after CD8+ T cell depletion in macaques infected with simian immunodeficiency virus [9] lended credence to the idea that virus specific CD8+ T cell immune responses are paramount for HIV suppression and subsequent HIV vaccines developed have since sought to induce potent virus specific cytotoxic CD8+ T cell immune responses.

## DNA vaccines against HIV

DNA mediated immunization or DNA vaccines represent the third generation approach to vaccinology. DNA vaccines are circular DNA molecules encoding a gene of interest that can be propagated in bacteria and injected / transfected into a host / cell line so that the encoded gene of interest is expressed in mammalian cells (by means of a strong viral promoter) and provokes a good immune response conferring immunity against the particular pathogen. The preclinical immunogenicity and/or efficacy of DNA vaccines in disease models of infectious diseases, cancer, allergy and autoimmune diseases have been demonstrated by a number of research groups [10-12].

There are several points to be considered while developing a DNA vaccine candidate. Early DNA vaccines such as pCMV plasmids consisted merely a DNA backbone and a promoter that would cause high expression levels of protein antigen in mammalian cells. However comparison of routes of delivery of these plasmids into rodents or humans have revealed that intramuscular delivery of the DNA yield the highest titer values. Thus muscle targeting vectors have gained more popularity [13-19].

DNA vaccines have also been designed to contain epitopes which direct the immune response of the host towards cellular or humoral immunity [20-22]. For example, direct injections of cDNA expression vectors encoding interleukin 2 (IL-2), IL-4, or type P1 transforming growth factor (TGF- $\beta$ 31) into mouse skeletal muscle induced biological effects characteristic of these cytokines *in vivo*. But amongst all the cytokines injected intramuscularly, the vector encoding IL-2 had enhanced humoral and cellular immune responses to an exogenous antigen [23,24]. A safety consideration in designing vaccines for humans is the transforming potential of the gene of interest. For example, the E6 and E7 proteins of the human papilloma virus are excellent targets for vaccination but also transform primary human epithelial cells *in vitro*, raising the possibility that they could do so *in vivo* [25].

The first HIV DNA vaccine using the envelope protein gene gp160 was tested in mice in 1993 and was shown to generate antibodies towards HIV [26]. In 1995, the same strategy was tested in monkeys and chimpanzees and both humoral and specific cellular immune responses were also observed. Importantly, intramuscular vaccination with DNA plasmids expressing HIV-1 genes decreased HIV-1 viral load in HIV-1-infected chimpanzees. In addition, naive (i.e. non-HIV-1-infected)

chimpanzees were protected against a heterologous challenge with HIV-1 [27]. These constructs were also used in humans [28].

In addition to inoculation with plasmid DNA containing genes targeting HIV alone, other vaccination regimens which included inoculation with multiple DNA plasmids encoding different genes or DNA plasmids in combination with recombinant cytokines [29], adjuvants of recombinant HIV specific proteins [30], booster doses, other plasmids containing expression cassettes of cytokines [31] started being followed. All these regimens sought to augment the CTL responses of the DNA vaccine.

### Vaccines based on viral backbones

The Merck STEP study used adenoviral vectors, which were strains that were made replication defective by mutations and deletions of the adenoviral genome. HIV constructs are then inserted in place of the deleted adenovirus genes and an exogenous promoter controls their expression. Two main adenoviral vectors have been tried. The NIH Vaccine Research Center (VRC) vector (serogroup 5, Ad5) expresses HIV gag and pol from clade B and env from clades A, B, and C; while the Merck MRKAd5 vector is a compilation of 3 adenoviruses that express gag, pol, and nef from clade B alone. In 2003, phase I human trials concluded the vaccine to be safe. However a phase IIb study showed that the vaccine induced the appropriate CTL responses but neither decreased viral load nor rates of infection [32,33].

Following the failure of the STEP trial, focus shifted to using other viral backbones such as the canarypox virus to deliver the HIV antigen. Canary pox virus is an avian virus that undergoes an abortive replication cycle in humans. Several canarypox virus carrying different HIV proteins have been tested. This vector induced antibody and CTL responses, but these responses were relatively weak and short-lived. A number of pre-trials with various subtype B canarypox-HIV vector primes and boosters containing subunit glycoprotein 120 or 160 (gp120 or gp160) first established the prime-boost concept as a candidate for advanced testing [30,34-36]. These canarypox-based prime-boost regimens induced both cellular and humoral responses, but CD8+ responses on enzyme-linked immunosorbent spot (ELISPOT) assay were low [34] and the presence of primary isolate neutralizing antibody was not consistently detected [35-39]. Since the STEP trial had demonstrated higher responses, this approach was abandoned initially.

However reduction in HIV acquisition by combination of a canarypox and DNA vaccine was established by the ALVAC trials in Thailand. The RV144 trial consisted of ALVAC-HIV (vCP1521), a recombinant canarypox vaccine developed by Virogenetics Corporation (Troy, NY) and manufactured by sanofi pasteur (Marcy-l'Étoile, France) where canarypox vector was genetically engineered to express HIV-1 Gag and Pro (subtype B LAI strain) and CRF01\_AE (subtype E) HIV-1 gp120 (92TH023) linked to the transmembrane 3 anchoring portion of gp41 (LAI) and a DNA vaccine, AIDSVAX B/E RV144 carrying the gp120 gene and [40,41]. This vaccine regimen demonstrated 31% efficacy in modified intention-to-treat analysis, which was the first time any efficacy has been demonstrated in humans.

The efficacy was highest early on and in those at lowest risk for HIV infection. The reason behind decline in vaccine efficacy over the first year after vaccination or the greater efficacy of the vaccine in persons at lower risk for infection could not be elucidated.

Furthermore the results were surprising because instead of a classical CTL response, the vaccine induced a CD4+ T cell and weakly neutralizing antibody response. The T-cell-line adapted neutralizing antibody (71% with response), antibody-directed-cell-mediated-cytotoxicity, and CD4+ lymphoproliferation (61% in response to gp20, 63% in response to gp120) have not been completely explained. Further individuals with higher plasma concentrations of immunoglobulin G (IgG) antibodies specific for the V1V2 loop of gp120 had lower rates of HIV infection, while high levels Env-specific IgA antibodies directly correlated with HIV infection [42].

### Current HIV vaccine research

As mentioned earlier, following HIV infection, there is a "normal" humoral response which is strain specific and causes the virus to mutate to evade the host immune response. 10-30% subjects develop broadly neutralizing antibodies and 1% of these subjects (elite neutralizers) then retain these at high enough titers for long enough periods to prevent progression of HIV infection to AIDS. Thus many studies have focused on identifying the antibodies from subjects in the elite group [43]. This has led to looking at immune therapy for suppression of HIV infection. For example a cocktail of 3 neutralizing antibodies administered to rhesus monkeys have decreased the viral titer in 7 days [44]. Vaccines eliciting a broad neutralizing antibody response are also being designed. Studies using other viral backbones which elicit broad humoral responses such as the Sendai virus [45], Vesicular stomatitis virus [46] has also been reported.

Other approaches include testing orally administered vaccines which do not trigger an immune response at all but prevent infection through activation of CD8+ regulatory T cells [47].

### Future Directions

An effective HIV-1 vaccine remains one of the highest research priorities and would have a significant public health impact. The scientific challenges to attain this goal nonetheless remain as of now.

### References

1. Cloyd MW, Chen JJ, Adegboyega P, Wang L (2001) How does HIV cause depletion of CD4 lymphocytes? A mechanism involving virus signaling through its cellular receptors. *Curr Mol Med* 1(5): 545-550.
2. Murphy KM, Stockinger B (2010) Effector T cell plasticity: flexibility in the face of changing circumstances. *Nat Immunol* 11(8): 674-680.
3. van Leeuwen EMM, Sprent J, Surh CD (2009) Generation and maintenance of memory CD4(+) T Cells. *Curr Opin Immunol* 21(2): 167-172.
4. Berman PW, Gregory TJ, Riddle L, Nakamura GR, Champe MA, et al. (1990) Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160.

- Nature 345(6276): 622-625. Belshe RB, Graham BS, Keefer MC, Gorse GJ, Wright P, et al. (1994) Neutralizing antibodies to HIV-1 in seronegative volunteers immunized with recombinant gp120 from the MN strain of HIV-1. NIAID AIDS Vaccine Clinical Trials Network. JAMA 272(6): 475-480.
5. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, et al. (2005) Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 191(5): 654-665.
  6. Connor RI, Korber BT, Graham BS, Hahn BH, Ho DD, et al. (1998) Immunological and Virological Analyses of Persons Infected by Human Immunodeficiency Virus Type 1 while Participating in Trials of Recombinant gp120 Subunit Vaccines. J Virol 72(2): 1552-1576.
  7. Musey L, Hughes J, Schacker T, Shea T, Corey L, et al. (1997) Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection. N Engl J Med 337(18): 1267-1274.
  8. Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, et al. (1999) Dramatic Rise in Plasma Viremia after CD8+T cell depletion in Simian Immunodeficiency Virus-Infected Macaques. J Exp Med 189(6): 991-998.
  9. Liu M (2003) DNA vaccines: a review. J Int Med 253(4): 402-410.
  10. Donnelly JJ, Ulmer JB, Shiver JW, Liu MA (1997) DNA vaccines. Annu Rev Immunol 15: 617-648.
  11. Montgomery DL, Ulmer JB, Donnelly JJ, Liu MA (1997) DNA vaccines. Pharmacol Ther 74(2): 195-205.
  12. Srivastava IK, Liu MA (2003) Gene Vaccines. Annals of Intern Med 138(7): 550-559.
  13. Corr M, von Damm A, Lee DJ, Tighe H (1999) *In vivo* priming by DNA injection occurs predominantly by antigen transfer. J Immunol 163(9): 4721-4727.
  14. Gurunathan S, Klinman DM, Seder RA (2000) DNA vaccines: immunology, application, and optimization. Annu Rev Immunol 18(1): 927-974.
  15. Davis HL, Mancini M, Michel ML, Whalen RG (1996) DNA-mediated immunization to hepatitis B surface antigen: longevity of primary response and effect of boost. Vaccine 14(9): 910-915.
  16. Macklin M, McCabe D, McGregor MW, Neumann V, Meyer T, et al. (1998) Immunization of pigs with a particle mediated DNA-vaccine to influenza A virus protects against challenge with homologous virus. J Virol 72(2): 1491-1496.
  17. Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner PL, et al. (1993) Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 259(5102): 1745-1749.
  18. Forns X, Emerson SU, Tobin GJ, Mishahwar IK, Purcell RH, et al. (1999) DNA immunization of mice and macaques with plasmids encoding hepatitis C virus envelope E2 protein expressed intracellularly and on the cell surface. Vaccine 17(15-16): 1992-2002.
  19. Lowrie DB, Whalen RG (1999) DNA vaccines: methods and protocols. Springer Science & Business Media 29.
  20. Saltzman WM, Shen H, Brandsma JL (2006) DNA vaccines: methods and protocols. Springer Science & Business Media 127.
  21. Raz E, Watanabe A, Baird SM, Eisenberg RA, Parr TB, et al. (1993) Systemic immunological effects of cytokine genes injected into skeletal muscle. Proc Natl Acad Sci USA 90(10): 4523-4527.
  22. Ma D, Gao M, Li J, Ma C, Li G (2013) Construction of novel cytokine by fusion of chicken IL-2 signal peptide to mature chicken IL-15 and comparison of the adjuvant effects by DNA immunization against Eimeria challenge. Vet Immunol Immunopathol 156(1-2): 114-120.
  23. He X, Tsang TC, Zhang T, Luo P, Harris DT (2005) Antigen epitope-expressing cytokines for DNA immunization. Vaccine 23(16): 1966-1972.
  24. Roden RB, Ling M, Wu TC (2004) Vaccination to prevent and treat cervical cancer. Human Path 35(8): 971-982.
  25. Wang B, Ugen KE, Srikantan V, Agadjanyan MG, Dang K, et al. (1993) Gene inoculation generates immune responses against human immunodeficiency virus type 1. Proc Natl Acad Sci USA 90(9): 4156-4160.
  26. Wang B, Boyer J, Srikantan V, Ugen K, Gilbert L, et al. (1995) Induction of Humoral and Cellular Immune Responses to the Human Immunodeficiency Type 1 Virus in Nonhuman Primates by *in Vivo* DNA Inoculation. Virology 211(1): 102-112.
  27. Ugen KE, Nyland SB, Boyer JD, Vidal C, Lera L, et al. (1998) DNA vaccination with HIV-1 expressing constructs elicits immune responses in humans. Vaccine 16(19): 1818-1821.
  28. Barouch DH, Santra S, Schmitz JE, Kuroda MJ, Fu TM, et al. (2000) Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science 290(5491): 486-492.
  29. Russell ND, Graham BS, Keefer MC, McElrath MJ, Self SG, et al. (2007) Phase 2 study of an HIV-1 canarypox vaccine (vCP1452) alone and in combination with rgp120: negative results fail to trigger a phase 3 correlates trial. J Acquir Immune Defic Syndr 44(2): 203-212.
  30. Kim JJ, Nottingham LK, Wilson DM, Bagarazzi ML, Tsai A, et al. (1998) Engineering DNA vaccines via co-delivery of co-stimulatory molecule genes. Vaccine 16(19): 1828-1835.
  31. Robertson M, Mehrotra D, Fitzgerald D, Duerr A, Casimiro DR, et al. (2008) Efficacy Results from the STEP Study (Merck V520 Protocol 023/HVTN 502): A Phase II Test-of-Concept Trial of the MRKA5 HIV-1 Gag/Pol/Nef Trivalent Vaccine.
  32. Robertson M, Casimiro DR, De Rosa S, Dubey S, Kierstead L, et al. (2008) Immunological Characterization of Subjects from the STEP Study: A Phase IIB Test-of-Concept Trial of the MRKA5 HIV-1 Gag/Pol/Nef Trivalent Vaccine.
  33. Belshe RB, Gorse GJ, Mulligan MJ, Evans TG, Keefer MC, et al. (1998) Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. NIAID AIDS Vaccine Evaluation Group. AIDS 12(18): 2407-2415.
  34. Verrier F, Burda S, Belshe R, Duliege AM, Excler JL, et al. (2000) A human immunodeficiency virus prime-boost immunization regimen in humans induces antibodies that show interclade cross-reactivity and neutralize several X4-, R5-, and dualtropic clade B and C primary isolates. J Virol 74(21): 10025-10033.
  35. Nitayaphan S, Pitisuttithum P, Karnasuta C, Eamsila C, de Souza M, et al. (2004) Safety and immunogenicity of an HIV subtype B and E prime-boost vaccine combination in HIV-negative Thai adults. J Infect Dis 190(4): 702-706.
  36. The AIDS Vaccine Evaluation Group 022 Protocol Team (2001) Cellular and humoral immune responses to a canarypox vaccine containing human immunodeficiency virus type 1 env, gag, and pro in combination with rgp120. J Infect Dis 183(4): 563-570.
  37. Mascola JR, Snyder SW, Weislow OS, Belay SM, Belshe RB, et al. (1996) Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary

- isolates of human immunodeficiency virus type 1. *J Infect Dis* 173(2): 340-348.
38. Zolla-Pazner S, Xu S, Burda S, Duliege AM, Excler JL, et al. (1998) Neutralization of syncytium-inducing primary isolates by sera from human immunodeficiency virus (HIV)-uninfected recipients of candidate HIV vaccines. *J Infect Dis* 178(5): 1502-1506.
39. Thongcharoen P, Suriyanon V, Paris RM, Khamboonruang C, de Souza MS, et al. (2007) A phase 1/2 comparative vaccine trial of the safety and immunogenicity of a CRF01\_AE (subtype E) candidate vaccine: ALVAC-HIV (vCP1521) prime with oligomeric gp160 (92TH023/LAI-DID) or bivalent gp120 (CM235/SF2) boost. *J Acquir Immune Defic Syndr* 46(1): 48-55.
40. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, et al. (2009) Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. *N Engl J Med* 361(23): 2209-2220.
41. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, et al. (2012) Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* 366(14): 1275-1286.
42. Walker LM, Huber M, Doores KJ, Falkowska E, Pejchal R, et al. (2011) Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* 477(7365): 466-470.
43. Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, et al. (2013) Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* 503(7475): 224-228.
44. Ishii H, Matano T (2015) Development of an AIDS vaccine using Sendai virus vectors. *Vaccine pii: S0264-410X(15)01007-5*.
45. Fuchs JD, Frank I, Elizaga ML, Allen M, Frahm N, et al. (2015) First-in-Human Evaluation of the Safety and Immunogenicity of a Recombinant Vesicular Stomatitis Virus Human Immunodeficiency Virus-1 gag Vaccine (HVTN 090). *Open Forum Infect Dis* 2(3): ofv082.
46. Lu W, Chen S, Lai C, Guo W, Fu L, et al. (2012) Induction of CD8+ regulatory T cells protects macaques against SIV challenge. *Cell Rep* 2(6): 1736-1746.