Importance of neutralization sieve analyses when seeking correlates of HIV-1 vaccine efficacy

David C Montefiori*

Laboratory for AIDS Vaccine Research & Development; Department of Surgery; Duke University Medical Center; Durham, NC USA

This commentary describes a rationale for the use of breakthrough viruses from clinical trial participants to assess neutralizing antibodies as a correlate of HIV-1 vaccine efficacy. The rationale is based on principles of a genetic sieve analysis, where the 2 analyses may be cooperative for delineating neutralizing antibodies as a mechanistic correlate of protection.

The identification of immunologic correlates of protection[1-3] against HIV-1 is a major goal that would greatly facilitate HIV-1 vaccine development. This information would provide a basis for rational immunogen design and could be used to guide the selection of promising immunogens to advance through preclinical and clinical testing. It also has value for qualifying the expected potency of different lots of vaccine preparations and for predicting vaccine efficacy in populations where phase 3 trials did not take place. Multiple cellular and humoral immune responses are seen in infected individuals, and these responses provide a template for what should be possible to elicit with vaccines that aim to either control virus replication or prevent infection altogether.[4,5] Preventing virus acquisition is a high priority for a virus like HIV-1 that integrates genetically and persists despite robust host immune responses. In this regard, neutralizing antibodies (nAbs) are among the most promising responses to induce with HIV-1 vaccines because of their well-documented ability to block infection in nonhuman primate passive protection studies with simian immunodeficiency virus (SIV) and chimeric simian-human immunodeficiency virus (SHIV).[6-12] As suggested by results of the RV144 HIV-1 vaccine efficacy trial and subsequent correlates studies,[13-16] Fc receptor-mediated effector functions might be another mechanism by which antibodies can prevent HIV-1 infection.[17-19] Indeed, these findings from RV144 emphasize the need to consider multiple antiviral mechanisms when delineating antibody correlates of protection in HIV-1 vaccine efficacy trials.

The overall effectiveness of vaccine-elicited nAbs will depend on the magnitude and breadth of neutralization across a wide spectrum of antigenic variants within and between the major genetic subtypes (clades) and circulating recombinant forms (CRFs) of HIV-1 that dominate the epidemic in geographic regions where a vaccine is most needed.[20,21] Current vaccine immunogens induce very little nAb against most of these variants.[22-26] Nonetheless, studies of HIV-1 infected individuals show that most people are capable of making Abs that neutralize diverse variants across multiple clades.[27,28] Studies of monoclonal Abs from some of the best neutralizers have identified several highly conserved regions of vulnerability on the viral envelope glycoproteins (Env), and are providing a wealth of new information on the biological requirements for inducing broadly nAbs with vaccines, leading to promising new avenues for improved immunogen designs.[27-32] To gauge progress with new immunogens, several highly standardized and formally validated assays are available for rapid, high-throughput assessments of the magnitude and breadth of neutralization

in preclinical and clinical HIV-1 vaccine trials.[33-36] Considerable infrastructure exists to perform these assessments in laboratories that comply with Good Clinical Laboratory Practices (GCLP), which is important for regulatory agency approval.[37,38] Current capacity includes laboratories that serve the Collaboration for HIV Vaccine Discovery (CAVD, Bill and Melinda Gates Foundation), the International AIDS Vaccine Initiative (IAVI) and major networks sponsored by the US National Institutes of Health, including the HIV Vaccine Trials Network (HVTN), the Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-ID), and the Simian Vaccine Evaluation Units (SVEUs).

These laboratory efforts are further strengthened by the availability of well-characterized HIV-1 reference strains that allow standardized neutralization data sets to be compared across vaccine protocols.[36,39-41] Notably, the initial panels of reference strains left open key questions about the number and overall composition of strains that will be needed to adequately assess vaccine-elicited responses. Some of these questions are addressed in the design of a recently-described global panel of reference viruses that aims to be applicable to multiple vaccine platforms and clades of HIV-1 in different parts of the world.[42] These reference strains are useful for comparing nAb responses among different vaccine immunogens[43]; however, their suitability for delineating nAbs as a correlate of vaccine efficacy remains to be proven. One unanswered question is whether these reference strains, which were selected based on their neutralization profiles with plasma samples from chronic HIV-1 infection, adequately represent the spectrum of epitope variants that need to be targeted by vaccines. This concern is compounded by the inherent limitations of typical case-control analyses that rely on response variability in vaccine recipients,[44] not taking into account differences between vaccine and placebo groups, and where the number of infection cases can be relatively low, especially for more effective vaccines. Thus, the standard practice of assaying the vaccine strain(s) and a small number of heterologous reference strains using case-control serum/plasma samples

from vaccine recipients, as was done for RV144,[14] may lack power to detect nAbs as a correlate. Moreover, case-control studies do not distinguish between a measured immune response that is mechanistically responsible for protection vs. a response that is predictive but not a component of the protective mechanism.[3]

To increase the power for detecting nAbs as a correlate of vaccine efficacy, breakthrough viruses from infected vaccine and placebo recipients may be used to seek evidence that the vaccine-elicited nAbs selectively blocked transmission of certain variants. This approach is analogous to a genetic sieve analysis, which looks for features in the sequences of viruses from infected vaccine and placebo recipients that significantly differ relative to the vaccine sequences as evidence of a vaccine effect against certain variants.[45,46] Likewise, a neutralization "sieve" analysis compares the phenotypic properties of viruses from infected vaccine and placebo recipients in terms of their sensitivity to neutralization by pre-infection plasma/serum samples from vaccine recipients at a peak immunogenicity time point.[25] A positive correlation would be indicated if viruses from vaccine recipients are found to be significantly less sensitive to neutralization than viruses from placebo recipients. This outcome would be evidence that the vaccine-elicited nAbs selectively blocked transmission of the more sensitive viruses, implying a direct causal effect in mediating protection. Corroborating evidence would come from a genetic sieve analysis that successfully identifies genetic signatures that correlate with vaccine efficacy and can be shown to be responsible for the neutralization phenotype, as was done in a recent study of vaccine-mediated protection against simian immunodeficiency virus infection in nonhuman primates.[47]

This approach, though simple in principle, is not without challenges. Additional resources would be needed to generate high fidelity molecular clones of functional Env genes from the plasma of infected trial participants to create the virus reagents needed for current assay technologies.[48] In addition, viral diversification during early infection has the potential to compromise the quality of

the analysis if the diversification affects vaccine-targeted epitopes prior to sampling. HIV-1 accumulates fixed amino acid changes as the host immune response matures and drives multiple rounds of virus escape from cytotoxic T lymphocyte (CTL) and nAb responses.[49,50] Although this immune pressure starts early in infection, the initial autologous nAb response is delayed and has a very narrow epitope specificity in any single individual,[49,51] possibly explaining why little diversification with potential to affect most antibody epitopes is seen in Env during the first 3–6 mo of infection.[52] Notably, a 6-mo sampling interval did not prevent the identification of a statistically significant genetic sieve effect in RV144.[45] A 6-mo sampling interval also did not prevent the identification of a significant genetic sieve effect in the STEP trial of a HIV-1 Gag, Pol, Nef vaccine that aimed to elicit protective CTL,[46] a finding that was possible under this condition even though the vaccine showed no clinical evidence of efficacy.[53] Although it remains possible that additional and stronger genetic sieve effects would have been detected if samples were obtained more frequently to capture the virus at earlier stages of infection, a sampling interval of no longer than 6 mo seems useful and has proven practical for an acceptable rate of compliance in large clinical trials.

Trial participants who acquire multiple variants at the time of transmission are another possible confounding factor for the neutralization sieve analysis. Current estimates of the rates of multiple variant transmissions are 19% for heterosexually acquired infections,[54-56] 36% for men who have sex with men,[56] and 42% for intravenous drug users (Katie Bar and George Shaw, personal communication). Extra care may be needed to identify these subjects and to include their multiple virus variants in the analysis.

Despite several challenges, neutralization sieve analyses with breakthrough viruses from vaccine and placebo recipients afford important advantages that merit serious consideration for highly variable viruses such as HIV-1. This is likely to be a more powerful method to detect nAb as a correlate of HIV-1 vaccine efficacy than methods that use the

vaccine strain(s) and heterologous reference strains. Moreover, phenotypic sieve analyses provide insights into whether the correlate is mechanistically responsible for protection.[3] A neutralization sieve analysis was performed for the Vax004 HIV-1 vaccine efficacy trial of a bivalent gp120 immunogen, in which a non-significant trend was seen toward a lower rate of infection in higher risk vaccine recipients,[57] and where several antibody measurements have suggested a weak vaccine effect on HIV-1 acquisition.[58,59] In that sieve analysis, pre-infection plasma samples obtained at a peak immunogenicity time point from 85 vaccine recipients were assayed against 13 breakthrough viruses from vaccine recipients and 14 breakthrough viruses from placebo recipients.[25] The results showed that the vaccine-elicited antibodies in Vax004 were more likely to neutralize viruses from placebo recipients than viruses from vaccine recipients (P = 0.004), suggesting the vaccine selectively blocked transmission of certain variants. A similar neutralization sieve analysis would be worthwhile for the RV144 trial, where modest but statistically significant protection was seen against HIV-1 acquisition,[13] and where plasmas from the primary immunogenicity time point exhibit neutralizing activity against many heterologous circulating strains of HIV-1 in an ultrasensitive assay[26] (additional unpublished observations).

Breakthrough viruses could be used in a similar fashion to assess other potential antiviral Ab activities as correlates of vaccine efficacy, including Fc receptor-mediated and complement-mediated effector functions. Moreover, in the event that initial case-control analyses identify multiple correlates of protection, follow-up studies with breakthrough viruses could be used to determine which correlate is most likely to be mechanistically responsible for protection and therefore more important for vaccine design, testing, regulatory approval, and quality assurance. Overall it would seem prudent to plan for these types of analysis in future efficacy trials of candidate HIV-1 vaccines.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Qin L, Gilbert PB, Corey L, McElrath MJ, Self SG. A framework for assessing immunological correlates of protection in vaccine trials. J Infect Dis 2007; 196:1304-12; PMID:17922394; http://dx.doi.org/10.1086/522428

2. Plotkin SA. Vaccines: correlates of vaccine-induced immunity. Clin Infect Dis 2008; 47:401-9; http://dx.doi.org/10.1086/589862; PMID:18558875

3. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis 2012; 54:1615-7; http://dx.doi.org/10.1093/cid/cis238; PMID:22437237

4. Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. Annu Rev Immunol 2010; 28:413-44; PMID:20192810; http://dx.doi.org/10.1146/annurev-immunol-030409-101256

5. McMichael AJ. HIV vaccines. Annu Rev Immunol 2006; 24:227-55; PMID:16551249; http://dx.doi.org/10.1146/annurev.immunol.24.021605.090605

6. Shibata R, Igarashi T, Haigwood N, Buckler-White A, Ogert R, Ross W, Willey R, Cho MW, Martin MA. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nat Med 1999; 5:204-10; http://dx.doi.org/10.1038/5568; PMID:9930869

7. Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, Hanson CE, Beary H, Hayes D, Frankel SS, Birx DL, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000; 6:207-10; http://dx.doi.org/10.1038/72318; PMID:10655111

8. Hessell AJ, Poignard P, Hunter M, Hangartner L, Tehrani DM, Bleeker WK, Parren PW, Marx PA, Burton DR. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. Nat Med 2009; 15:951-4; http://dx.doi.org/10.1038/nm.1974; PMID:19525965

9. Baba TW, Liska V, Hofmann-Lehmann R, Vlasak J, Xu W, Ayehunie S, Cavacini LA, Posner MR, Katinger H, Stiegler G, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat Med 2000; 6:200-6; PMID:10655110; http://dx.doi.org/10.1038/72309

10. Nishimura Y, Igarashi T, Haigwood N, Sadjadpour R, Plishka RJ, Buckler-White A, Shibata R, Martin MA. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. J Virol 2002; 76:2123-30; PMID:11836389; http://dx.doi.org/10.1128/jvi.76.5.2123-2130.2002

11. Nishimura Y, Igarashi T, Haigwood NL, Sadjadpour R, Donau OK, Buckler C, Plishka RJ, Buckler-White A, Martin MA. Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. Proc Natl Acad Sci U S A 2003; 100:15131-6; PMID:14627745; http://dx.doi.org/10.1073/pnas.2436476100

12. Ferrantelli F, Hofmann-Lehmann R, Rasmussen RA, Wang T, Xu W, Li PL, Montefiori DC, Cavacini LA, Katinger H, Stiegler G, et al. Post-exposure prophylaxis with human monoclonal antibodies prevented SHIV89.6P infection or disease in neonatal macaques. AIDS 2003; 17:301-9; PMID:12556683; http://dx.doi.org/10.1097/00002030-200302140-00003

13. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, et al.; MOPH-TAVEG Investigators. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009; 361:2209-20; http://dx.doi.org/10.1056/NEJMoa0908492; PMID:19843557

14. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 2012; 366:1275-86; http://dx.doi.org/10.1056/NEJMoa1113425; PMID:22475592

15. Gottardo R, Bailer RT, Korber BT, Gnanakaran S, Phillips J, Shen X, Tomaras GD, Turk E, Imholte G, Eckler L, et al. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. PLoS One 2013; 8:e75665; http://dx.doi.org/10.1371/journal.pone.0075665; PMID:24086607

16. Zolla-Pazner S, deCamp A, Gilbert PB, Williams C, Yates NL, Williams WT, Howington R, Fong Y, Morris DE, Soderberg KA, et al. Vaccine-induced IgG antibodies to V1V2 regions of multiple HIV-1 subtypes correlate with decreased risk of HIV-1 infection. PLoS One 2014; 9:e87572; http://dx.doi.org/10.1371/journal.pone.0087572; PMID:24504509

17. Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, Gilbert PB, Huang Y, Gurley TC, Kozink DM, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. J Virol 2012; 86:11521-32; http://dx.doi.org/10.1128/JVI.01023-12; PMID:22896626

18. Yates NL, Liao H-X, Fong Y, deCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang Z-Y, Seaton KE, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. Sci Transl Med. 2014; 6(228):228ra9

19. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, Dugast A-S, Schoen MK, Rolland M, Suscovich TJ, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. Sci Transl Med. 2014 Mar 19;6(228):228ra38

20. Hemelaar J, Gouws E, Ghys PD, Osmanov S; WHO-UNAIDS Network for HIV Isolation and Characterisation. Global trends in molecular epidemiology of HIV-1 during 2000-2007. AIDS 2011; 25:679-89; http://dx.doi.org/10.1097/QAD.0b013e328342ff93; PMID:21297424

21. Hemelaar J. Implications of HIV diversity for the HIV-1 pandemic. J Infect 2013; 66:391-400; http://dx.doi.org/10.1016/j.jinf.2012.10.026; PMID:23103289

22. Mascola JR, Snyder SW, Weislow OS, Belay SM, Belshe RB, Schwartz DH, Clements ML, Dolin R, Graham BS, Gorse GJ, et al.; The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. 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 1996; 173:340-8; PMID:8568294; http://dx.doi.org/10.1093/infdis/173.2.340

23. Bures R, Gaitan A, Zhu T, Graziosi C, McGrath KM, Tartaglia J, Caudrelier P, El Habib R, Klein M, Lazzarin A, et al. Immunization with recombinant canarypox vectors expressing membrane-anchored glycoprotein 120 followed by glycoprotein 160 boosting fails to generate antibodies that neutralize R5 primary isolates of human immunodeficiency virus type 1. AIDS Res Hum Retroviruses 2000; 16:2019-35; PMID:11153085; http://dx.doi.org/10.1089/088922200750054756

24. Belshe RB, Gorse GJ, Mulligan MJ, Evans TG, Keefer MC, Excler JL, Duliege AM, Tartaglia J, Cox WI, McNamara J, et al.; NIAID AIDS Vaccine Evaluation Group. Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. AIDS 1998; 12:2407-15; PMID:9875578; http://dx.doi.org/10.1097/00002030-199818000-00009

25. Gilbert P, Wang M, Wrin T, Petropoulos C, Gurwith M, Sinangil F, D'Souza P, Rodriguez-Chavez IR, DeCamp A, Giganti M, et al. Magnitude and breadth of a nonprotective neutralizing antibody response in an efficacy trial of a candidate HIV-1 gp120 vaccine. J Infect Dis 2010; 202:595-605; http://dx.doi.org/10.1086/654816; PMID:20608874

26. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, de Souza MS, McLinden R, Tovanabutra S, Laurence-Chenine A, Sanders-Buell E, et al. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. J Infect Dis 2012; 206:431-41; http://dx.doi.org/10.1093/infdis/jis367; PMID:22634875

27. Hraber P, Seaman MS, Bailer RT, Mascola JR, Montefiori DC, Korber BT. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. AIDS 2014; 28:163-9; http://dx.doi.org/10.1097/QAD.0000000000000106; PMID:24361678

28. Stamatatos L, Morris L, Burton DR, Mascola JR. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? Nat Med 2009; 15:866-70; PMID:19525964

29. Kwong PD, Mascola JR. Human antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies. Immunity 2012; 37:412-25; PMID:22999947; http://dx.doi.org/10.1016/j.immuni.2012.08.012

30. Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, Kaufmann DE, McElrath MJ, Nussenzweig MC, Pulendran B, et al. A Blueprint for HIV Vaccine Discovery. Cell Host Microbe 2012; 12:396-407; http://dx.doi.org/10.1016/j.chom.2012.09.008; PMID:23084910

31. Corti D, Lanzavecchia A. Broadly neutralizing antiviral antibodies. Annu Rev Immunol 2013; 31:705-42; PMID:23330954; http://dx.doi.org/10.1146/annurev-immunol-032712-095916

32. Klein F, Mouquet H, Dosenovic P, Scheid JF, Scharf L, Nussenzweig MC. Antibodies in HIV-1 vaccine development and therapy. Science 2013; 341:1199-204; PMID:24031012; http://dx.doi.org/10.1126/science.1241144

33. Montefiori DC. Measuring HIV neutralization in a luciferase reporter gene assay. Methods Mol Biol 2009; 485:395-405; PMID:19020839; http://dx.doi.org/10.1007/978-1-59745-170-3_26

34. Sarzotti-Kelsoe M, Bailer RT, Turk E, Lin CL, Bilska M, Greene KM, Gao H, Todd CA, Ozaki DA, Seaman MS, et al. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. J Immunol Methods 2013; http://dx.doi.org/10.1016/j.jim.2014.02.013, In press; PMID:24291345.

35. Sarzotti-Kelsoe M, Daniell X, Todd CA, Bilska M, Martelli A, Labranche C, Perez LG, Ochsenbauer C, Kappes JC, Rountree W, et al. Optimization and validation of a neutralizing antibody assay for HIV-1 in A3R5 cells. J Immunol Methods 2014; In press; PMID:24607608; http://dx.doi.org/10.1016/j.jim.2014.02.013.

36. Simek MD, Rida W, Priddy FH, Pung P, Carrow E, Laufer DS, Lehrman JK, Boaz M, Tarragona-Fiol T, Miiro G, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J Virol 2009; 83:7337-48; PMID:19439467; http://dx.doi.org/10.1128/JVI.00110-09

37. Todd CA, Greene KM, Yu X, Ozaki DA, Gao H, Huang Y, Wang M, Li G, Brown R, Wood B, et al. Development and implementation of an international proficiency testing program for a neutralizing antibody assay for HIV-1 in TZM-bl cells. J Immunol Methods 2012; 375:57-67; http://dx.doi.org/10.1016/j.jim.2011.09.007; PMID:21968254

38. Ozaki DA, Gao H, Todd CA, Greene KM, Montefiori DC, Sarzotti-Kelsoe M. International technology transfer of a GCLP-compliant HIV-1 neutralizing antibody assay for human clinical trials. PLoS One 2012; 7:e30963; PMID:22303476; http://dx.doi.org/10.1371/journal.pone.0030963

39. Li M, Gao F, Mascola JR, Stamatatos L, Polonis VR, Koutsoukos M, Voss G, Goepfert P, Gilbert P, Greene KM, et al. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. J Virol 2005; 79:10108-25; http://dx.doi.org/10.1128/JVI.79.16.10108-10125.2005; PMID:16051804

40. Li M, Salazar-Gonzalez JF, Derdeyn CA, Morris L, Williamson C, Robinson JE, Decker JM, Li Y, Salazar MG, Polonis VR, et al. Genetic and neutralization properties of subtype C human immunodeficiency virus type 1 molecular env clones from acute and early heterosexually acquired infections in Southern Africa. J Virol 2006; 80:11776-90; http://dx.doi.org/10.1128/JVI.01730-06; PMID:16971434

41. Brown BK, Darden JM, Tovanabutra S, Oblander T, Frost J, Sanders-Buell E, de Souza MS, Birx DL, McCutchan FE, Polonis VR. Biologic and genetic characterization of a panel of 60 human immunodeficiency virus type 1 isolates, representing clades A, B, C, D, CRF01_AE, and CRF02_AG, for the development and assessment of candidate vaccines. J Virol 2005; 79:6089-101; http://dx.doi.org/10.1128/JVI.79.10.6089-6101.2005; PMID:15857994

42. deCamp A, Hraber P, Bailer RT, Seaman MS, Ochsenbauer C, Kappes J, Gottardo R, Edlefsen P, Self S, Tang H, et al. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. J Virol 2014; 88:2489-507; http://dx.doi.org/10.1128/JVI.02853-13; PMID:24352443

43. Mascola JR, D'Souza P, Gilbert P, Hahn BH, Haigwood NL, Morris L, Petropoulos CJ, Polonis VR, Sarzotti M, Montefiori DC. Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by candidate human immunodeficiency virus type 1 vaccines. J Virol 2005; 79:10103-7; http://dx.doi.org/10.1128/JVI.79.16.10103-10107.2005; PMID:16051803

44. Rolland M, Gilbert P. Evaluating immune correlates in HIV type 1 vaccine efficacy trials: what RV144 may provide. AIDS Res Hum Retroviruses 2012; 28:400-4; http://dx.doi.org/10.1089/aid.2011.0240; PMID:21902593

45. Rolland M, Edlefsen PT, Larsen BB, Tovanabutra S, Sanders-Buell E, Hertz T, deCamp AC, Carrico C, Menis S, Magaret CA, et al. Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. Nature 2012; 490:417-20; http://dx.doi.org/10.1038/nature11519; PMID:22960785

46. Rolland M, Tovanabutra S, deCamp AC, Frahm N, Gilbert PB, Sanders-Buell E, Heath L, Magaret CA, Bose M, Bradfield A, et al. Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. Nat Med 2011; 17:366-71; http://dx.doi.org/10.1038/nm.2316; PMID:21358627

47. Roederer M, Keele BF, Schmidt SD, Mason RD, Welles HC, Fischer W, Labranche C, Foulds KE, Louder MK, Yang ZY, et al. Immunological and virological mechanisms of vaccine-mediated protection against SIV and HIV. Nature 2014; 505:502-8; http://dx.doi.org/10.1038/nature12893; PMID:24352234

48. Salazar-Gonzalez JF, Bailes E, Pham KT, Salazar MG, Guffey MB, Keele BF, Derdeyn CA, Farmer P, Hunter E, Allen S, et al. Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing. J Virol 2008; 82:3952-70; http://dx.doi.org/10.1128/JVI.02660-07; PMID:18256145

49. Bar KJ, Tsao CY, Iyer SS, Decker JM, Yang Y, Bonsignori M, Chen X, Hwang KK, Montefiori DC, Liao HX, et al. Early low-titer neutralizing antibodies impede HIV-1 replication and select for virus escape. PLoS Pathog 2012; 8:e1002721; http://dx.doi.org/10.1371/journal.ppat.1002721; PMID:22693447

50. Goonetilleke N, Liu MK, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov VV, Keele BF, Learn GH, Turnbull EL, Salazar MG, et al.; CHAVI Clinical Core B. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J Exp Med 2009; 206:1253-72; http://dx.doi.org/10.1084/jem.20090365; PMID:19487423

51. Moore PL, Gray ES, Morris L. Specificity of the autologous neutralizing antibody response. Curr Opin HIV AIDS 2009; 4:358-63; http://dx.doi.org/10.1097/COH.0b013e32832ea7e8; PMID:20048698

52. Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, Decker JM, Wang S, Baalwa J, Kraus MH, et al. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. J Exp Med 2009; 206:1273-89; http://dx.doi.org/10.1084/jem.20090378; PMID:19487424

53. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gilbert PB, Lama JR, Marmor M, Del Rio C, et al.; Step Study Protocol Team. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008; 372:1881-93; http://dx.doi.org/10.1016/S0140-6736(08)61591-3; PMID:19012954

54. Haaland RE, Hawkins PA, Salazar-Gonzalez J, Johnson A, Tichacek A, Karita E, Manigart O, Mulenga J, Keele BF, Shaw GM, et al. Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1. PLoS Pathog 2009; 5:e1000274; http://dx.doi.org/10.1371/journal.ppat.1000274; PMID:19165325

55. Abrahams MR, Anderson JA, Giorgi EE, Seoighe C, Mlisana K, Ping L-H, Athreya GS, Treurnicht FK, Keele BF, Wood N, et al.; CAPRISA Acute Infection Study Team; Center for HIV-AIDS Vaccine Immunology Consortium. Quantitating the multiplicity of infection with human immunodeficiency virus type 1 subtype C reveals a non-poisson distribution of transmitted variants. J Virol 2009; 83:3556-67; http://dx.doi.org/10.1128/JVI.02132-08; PMID:19193811

56. Li H, Bar KJ, Wang S, Decker JM, Chen Y, Sun C, Salazar-Gonzalez JF, Salazar MG, Learn GH, Morgan CJ, et al. High multiplicity infection by HIV-1 in men who have sex with men. PLoS Pathog. 2010 May 13;6(5):e1000890

57. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF; rgp120 HIV Vaccine Study Group. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 2005; 191:654-65; PMID:15688278; http://dx.doi.org/10.1086/428404

58. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, Heyward WL, Jobes DV, Popovic V, Self SG, et al. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis 2005; 191:666-77; PMID:15688279; http://dx.doi.org/10.1086/428405

59. Forthal DN, Gilbert PB, Landucci G, Phan T. Recombinant gp120 vaccine-induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. J Immunol 2007; 178:6596-603; PMID:17475891; http://dx.doi.org/10.4049/jimmunol.178.10.6596