Journal of AIDS and HIV Infections
Volume 6 | Issue 1
ISSN: 2455-7633

Review Article Open Access

The Argument for Thoughtful Empiricism in AIDS Vaccine Development

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Received Date: May 22, 2021 **Accepted Date:** June 24, 2021 **Published Date:** June 26, 2021

Citation: Burton P. Dorman (2021) The Argument for Thoughtful Empiricism in AIDS Vaccine Development. J Aids Hiv Inf 6(1): 103

Abstract

History teaches that successful vaccine development is a highly empirical process involving guesswork and tinkering, typically done in the face of woefully incomplete biological understanding. The AIDS vaccine effort effectively abandoned this classical approach early on. It embraced instead an ambitious program of basic research designed to unravel underlying biological mysteries and to lead eventually to "rational design" of innovative vaccines based upon well-understood biological principals. Thinking our way to a better vaccine is an appealing idea. But basic research is not famous for near-term solutions to practical problems. Several decades, tens of millions of lives, and billions of research dollars later, we have no vaccine in sight, and no way to tell how near we might be-- or how far. In retrospect, the basic research effort could have been augmented, and arguably should have been, by a no less diligent classical trial-and-error vaccinology undertaking, employing thoughtful empiricism focused on proven methods like killed-virus vaccines. Killed-virus methods have produced clinically useful outcomes in several animal retrovirus models. This classical approach may be able to protect humans but never was investigated adequately to rule it in or out for HIV. It likely can be tested definitively for human safety/efficacy in five years or less-- perhaps far sooner than anyone will invent something better. And, if proven to be safe and effective, this approach potentially can salvage prospects for a reasonable life for millions of people who otherwise will fall victim to AIDS. So it should be tested.

Keywords: AIDS vaccine

Text

The U. S. National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), announced in early February, 2020 the need to interrupt the "Uhambo" AIDS vaccine trial (HVTN 702) begun in South Africa in 2016. Interim analysis by an independent review panel had determined conclusively that the six-injection vaccination regimen totally failed to prevent AIDS [1].

It is almost impossible to overstate just how thoroughly disappointing was the failure of this trial [2]. The roots of HVTN 702 can be traced to research and development efforts initiated near the very outset of the AIDS pandemic almost 40 years ago. Contributions were drawn from prestigious elements of academia, advocacy groups, biotech firms, pharmaceutical companies, government and philanthropic entities. Their work produced a series of candidate vaccine formulations tested in clinical trials in the 1990s and extending through the subsequent two decades. HVTN 702 was modeled specifically on the Thai trial (RV144) run between 2006 and 2009-- the only vaccine trial to date yielding any sign of efficacy for prevention of AIDS [3]. We knew, of course, that HVTN 702 might not succeed; that is why we run clinical trials. Still, there was ample reason to view this as perhaps our most promising near term shot at an effective prophylactic vaccine. The disappointing outcome compels careful review of the AIDS vaccine research effort to date, why it was framed as a basic research undertaking aimed at unraveling the extremely complex underlying biology, why classical vaccine methods and empirical trial-and-error methodology were abandoned in favor of more-innovative vaccine approaches, and why at long last it arguably is time to reconsider use of classical vaccinology and proven methods like killed-virus vaccines.

In the beginning

AIDS vaccine development history effectively began on April 23, 1984 when Margaret Heckler, then U.S. Secretary of Health and Human Services, held a press conference to announce that a retrovirus had been identified as the cause of AIDS. She relayed the assessment of her science advisors that a vaccine could be ready for human testing in two to three years [4]. This prediction has garnered much ridicule over the intervening decades. But, given the assumption that, in a matter this urgent, we would examine initially those vaccine methods already proven to be safe and effective in other contexts, it was reasonable to suppose that one or more vaccine candidates might be ready for human testing quite promptly.

At that time, with only a single exception, all vaccines for viral disease used one of the two available "classical" methods. "Live attenuated" virus vaccines employed live virus that retains the ability to infect but is weakened (attenuated) so as not to cause illness. This method is used for many human viral diseases, including measles, mumps, rubella, chickenpox, polio (Sabin), shingles, smallpox, and yellow fever. The second classical method used killed virus-- formerly live virus that has been inactivated ("killed") during vaccine manufacture to eliminate its ability to infect. "Killed-virus" vaccines are widely employed for hepatitis A, flu, polio (Salk), and rabies.

But by the time AIDS emerged, we also had access to a novel method that utilized newly invented recombinant DNA techniques to make "recombinant protein subunit" vaccines. This approach had recently been employed successfully for hepatitis B and had attracted widespread interest among both academic and private-sector scientists-- and for good reason. A novel, innovative method represented "cutting edge" science, so it was far more likely to attract research support for academic investigators. And it was much more apt to produce proprietary patents and profits for commercial research entities.

Understandably, therefore, researchers interested in AIDS vaccines found greater incentive to focus on approaches that drew upon these modern methods. Unfortunately, results from early clinical trials of several recombinant vaccine candidates were not highly encouraging. In the absence of promising outcomes based on known methods, almost every researcher, and every granting agency, was quite willing to embrace the fateful 1994 *Nature* commentary titled "AIDS: time to turn to basic science" authored by respected

Harvard virologist Bernard Fields [5]. He argued that "these familiar paradigms have not shown great promise" for HIV/AIDS, and thus we "must return to a broader base," namely basic science, to discover new ones.

Unraveling biological mysteries: "the thinker hypothesis"

Basic science, although it is inestimably valuable, is not famous for producing near term solutions to practical problems. Nonetheless, by 2006, the basic research endeavor had evolved into an even more ambitious aspiration-- to obtain a vaccine through "rational design." The declared goal of the rational design enterprise was "to develop well-understood scientific principles that predict protective immune responses" [6,7].

One influential viewpoint, proposed in 2009, was to argue that HIV/AIDS would require a vaccine able to "do better than nature" and that this would necessitate "a shift in the balance of effort from the empirical development and large-scale testing of candidate vaccines to discovery of the mysteries of interaction of HIV and the body's immune system" [8]. This effectively became the governing paradigm for the most recent decade of AIDS vaccine research.

In a thoughtful and painstaking effort to parse just which biological mysteries confronted us as of 2010, Virgin and Walker observed that "We still lack fundamental knowledge regarding the nature, quality, and quantity of immune responses" that would be needed or how to induce them. They described forty "knowable unknowns," each one a challenging biological question that might require explication en route to a clinically useful vaccine. It was acknowledged that many scientists thought a (rationally designed) vaccine "is probably decades away, if even possible" [9].

The 2010 "knowable unknown" that many researchers consider especially important was "How do we generate broadly cross-protective antibodies?" Eight years later, in 2018, when Subbaraman *et al.* examined current views on how to elicit broadly neutralizing antibodies (bnAbs), they identified 33 distinct biological parameters that appear to influence bnAb development and seven key undeciphered questions regarding bnAb development whose answers apparently will be needed to move HIV vaccine design forward [10]. Ironically, we have less than unanimous agreement that bnAbs are either necessary [11,12] or sufficient [13-15] to elicit protective immune responses.

If, in order to resolve such questions, we truly must rely upon basic research to unravel outstanding biological mysteries, then we still may be several decades from a clinically useful vaccine. The deeper we go into the biological details, the more we discover is still left to learn, and the more complex the mysteries appear to be [10,11,16,17] and see Figure. As of today, virtually every one of the "knowable unknowns" described in 2010 remains incompletely understood. "The immense complexity and multitude of unanswered questions remain enormous challenges which have to be overcome" [18]. Not everyone concedes that rational design is even feasible owing to the complex and uncertain relationship between chemical antigenicity and biological immunogenicity [19].

But even if the effort to resolve biological mysteries did lead to a clinically useful AIDS vaccine one day, we have no way to know how soon that day may come. The effort to discover and unravel biological mysteries does not readily lend itself to time lines or target dates. And this rational design endeavor, based on the unproven hypothesis that we can unravel the relevant biological mysteries and think our way to a vaccine, clearly represents a dramatic departure from the historically successful practice of vaccinology [20].

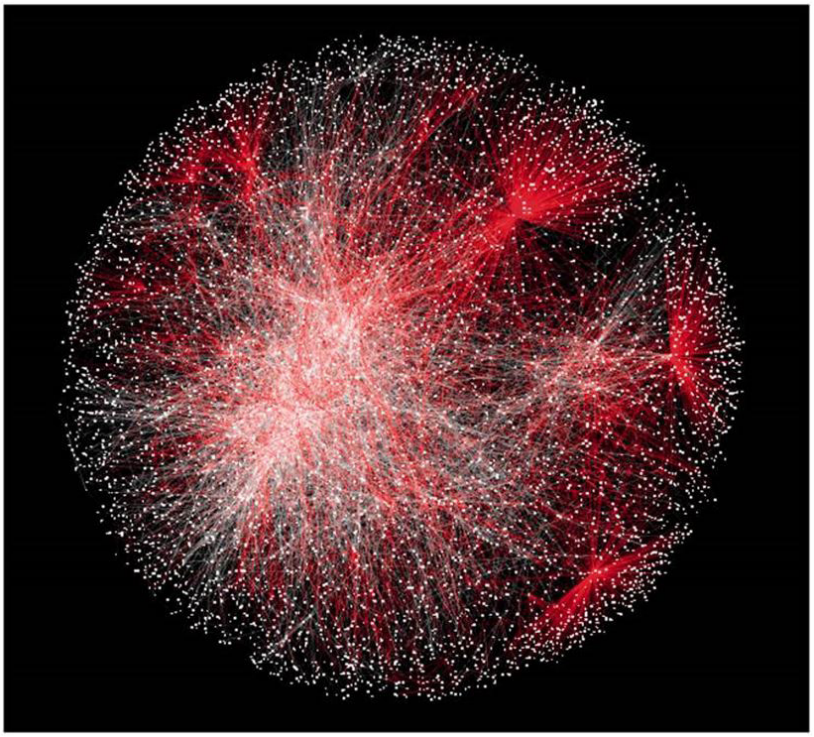

Figure: Graphic representation of the "daunting complexity" of the innate immune system

The white dots represent the 957 human genes of known relevance to human innate immunity. The lines between dots represent the >18,000 molecular interactions between their gene products which had been annotated as of 2012. For detailed statistics see http://www.innatedb.com/statistics. Adaptive immune system responses to a pathogen, not represented here, may be no less complex.

The "thoughtful empiricism" of classical vaccinology

Dating from the pioneering work of Edward Jenner in the late 18th century, vaccine development always had been a highly empirical undertaking done in the context of rather poorly understood biology. The basic idea was simple: to expose vaccinees to an immunogen that would not cause serious illness but that would trick their immune system into responding as if it had been infected by the pathogen. Once primed in this way, the immune response then might be faster and more effective in case the real infectious agent was to be encountered subsequently. For that reason, we all were taught that protective immune responses should be primed with vaccine immunogens that mimicked the circulating pathogen as closely as possible.

This traditional "mimic the pathogen" strategy is not guaranteed to work. It has been especially problematic for rapidly mutating pathogens like flu; it is even less promising for a retrovirus like HIV. This is so because a significant portion of the characteristic response to HIV infection is directed to viral features that are free to mutate very rapidly, so today's immune response typically is directed to yesterday's version(s) of the HIV pathogen. Without timely access to antiviral medication, healthy persons infected by HIV virtually always succumb to AIDS eventually-- despite prompt and robust *natural* immune responses. Therefore, it became clear early on that a successful AIDS vaccine might require immune responses that were different than typical responses to HIV infection [21]. The questions were, and still are, how to elicit more useful clinical outcomes, and how to do this sooner rather than later?

This problem is complicated by the historical observation that vaccination outcomes can be influenced by small changes in a sizable list of vaccine input parameters, any of which can spell the difference between success and failure. These include the virus strain and isolate selected, host cell system used to produce the virus, growth conditions, virus isolation and purification methods, plus myriad details of vaccine formulation and administration, *e.g.,* adjuvant, dosage, route of administration, and immunization schedule. Until proven otherwise, we must assume that *everything matters* and must identify a near-optimal value for each parameter. But little if any theoretical framework exists upon which to mount our choices. So we typically have required numerous guesses, considerable empirical screening, multiple iterative trials, and a series of small incremental improvements before we hit upon safe and effective formulations [22]. We have seen no evidence to suggest HIV might be an exception to this rule. Therefore, absent enormous good luck, we must be prepared to test large numbers of candidate vaccine formulations, and failure is likely to greet early trials of AIDS vaccines made by any method, including any of the vaccine candidate formulations now undergoing clinical evaluation. As has been noted, "we clearly need more 'shots on goal'" [23].

The AIDS vaccine problem is further complicated by the lack of a useful animal model for HIV infection. Therefore, meaningful screening of candidate vaccines must be conducted in the context of the human immune system [24]. Trials run in other species have not provided a reliable gauge for results of human efficacy trials [25]. But the number of HIV vaccine candidates that can be tested for efficacy in humans will remain limited by various factors-- biological, economic, medical, sociological, and ethical. So the obvious questions are: (a) how to screen a far larger number of vaccine candidates, (b) how to do that more quickly and more economically, and (c) how to make better-informed choices about selection of vaccine formulations that should be advanced to lengthy and expensive human efficacy trials.

Haynes Sheppard and I have proposed that these questions are best addressed using systematic, head to head comparison of candidate vaccine formulations in microtrials of human immunogenicity. We would perform these microtrials early in the product development cycle-- as soon as compatible with safety [26]. If needed, a large number of candidate vaccine formulations could be compared promptly and relatively economically. Which responses would lead to protective efficacy remains unclear. However, in contrast to any tests run in other species, assessment of their human immunogenicity would generate better informed choices of which vaccine formulations warrant advancement to efficacy testing-- regardless of which vaccine method one might choose to investigate.

In essence, this strategy represents a return to the thoughtful trial-and-error empiricism of classical vaccinology-- an approach that always has been readily available, although it never yet has been adequately exploited for HIV. The entire point of classical vaccinology is to get something useful-- and to get it as soon as possible. As explained by Maurice Hilleman, history's most prolific vaccinologist: "Success in vaccinology depends on simplification of the complex.... By definition, a vaccinologist might be considered a reductionist who pursues the simple and the practical in a universe of theory and complexity" [24]. This formula sounds like standard engineering practice. So, it should be no surprise that contemporary computer software engineers advocate essentially the identical strategy: "Begin by solving the simplest version of the problem. With each increment of an iterative development, do the simplest thing that could possibly work" [27].

Arguments for and against killed HIV-- the simplest thing that could possibly work

A few of us have proposed that the simplest thing with realistic potential to yield safe, effective AIDS vaccines very promptly would be to reframe a piece of the research endeavor as a problem in classical, highly empirical, trial-and-error vaccinology [26,28,29]. Specifically, we would augment the current HIV/AIDS vaccine enterprise with a systematic product development effort focused on killed-virus vaccines modeled after existing efficacious vaccine products, including killed animal retrovirus vaccines and/or killed-virus vaccines for human polio, rabies, or influenza [26].

Vaccines made using killed virus will need to confront and overcome two daunting challenges: (1) the genomic variation associated with the characteristic high mutation rate of all retrovirus, and (2) the intrinsic risk posed by the ability of retrovirus to insert their

genetic material into chromosomes of the infected host during even a brief "transient" infection. Given a choice, we clearly would prefer a vaccine able to prevent even a very short-lived infection. However, the issue posed by HIV's extraordinary genetic diversity must be confronted by every vaccine approach-- bar none. And, as yet, no viral vaccine made by any method has demonstrated the ability to unequivocally prevent even a transient infection. But killed virus vaccines have deferred and/or reduced severity of illness and inhibited transmission of diseases caused by animal retroviruses closely related to HIV [30-33]. Not one experimental or clinical observation has yet been reported to rule out the possibility that killed-HIV vaccines might produce comparable outcomes for humans. It has been acknowledged that even a moderately efficacious vaccine would significantly advance prospects to interrupt the AIDS pandemic [34]. So the possibility that one day we may discover more ideal vaccine methods is not good reason to forego the benefit that might be available far sooner if we diligently investigated methods which already have been invented, in particular killed virus vaccines. Until we discover how to make something better, even a costly killed-virus vaccine would be preferable to no vaccine. And if it were safe and efficacious, however costly, we would expect to derive crucial information on "correlates of protection"-- which potentially would be an invaluable aid to subsequent development of improved, next-generation products using more innovative approaches.

As others have suggested, it could be particularly desirable for a vaccine to elicit potent responses shifted *away* from HIV's normally immunodominant variable epitopes and *toward* more highly conserved viral structures, e.g., the CD-4 binding site [35,36]. Such shifts would not be expected from inactivation protocols designed to preserve the antigenic properties of native virions [37,38]. And we have no evidence as yet that clinically useful human responses can be elicited with any formulation or any presentation of immunogenically unmodified (native) viral envelope, such as those employed in the just-suspended HTVN 702 trial [1] or in the HVTN 705 [39] and HVTN 706 [40] efficacy trials currently underway in Africa and elsewhere.

However, historical observations demonstrate that potentially useful immunogenicity shifts can be achieved for killed-virus vaccines through selective epitope-specific chemical action of common virus inactivation agents [41-44]. As a practical matter, killed-virus vaccines made with some form of the classical chemical inactivant formaldehyde [30-33] represent the only method offering (1) demonstrated clinical efficacy for retroviral disease and (2) intrinsic risks that are sufficiently well understood to justify regulatory approval and informed consent for human microtrials early in the product development cycle.

But the killed-HIV vaccine approach never has received thorough, systematic, definitive evaluation. Why not? In principle, it was easy to imagine that a novel vaccine method might be better-- more potent, more efficacious, convenient, or economical to manufacture, store, or administer. There also were understandable concerns about safety drawn from the tragic 1955 "Cutter incident" and "Wyeth problem", when incompletely inactivated polio vaccine led to ~300 paralytic polio cases among vaccinated children and their contacts [45,46]. In practice, however, hundreds of millions of killed-virus vaccine doses have been administered safely worldwide since those events in 1955-- the last time a killed-virus vaccine is known to have transmitted disease from residual live virus. It has been acknowledged [47,48] and demonstrated [28] that combinations of conventional inactivation agents yield adequate safety margins for HIV and that safety issues can ultimately be addressed. Concerns about transmitting disease are rendered entirely moot if genetic material of the virus (and host cells) is removed, as readily can be done using a killed, split-virus vaccine à la flu. So it is worth repeating here: the argument that we might one day invent a safer, more effective method is not a good excuse for continued disregard of benefits that could be available far sooner from carefully manufactured killed-virus vaccines. And, currently, nothing better is in sight.

Meaningful potential impact

As the saying goes, “we all are victims of our experience.” Basic scientists clearly have demonstrated that we sometimes can think our way to new ideas that unravel important and consequential mysteries. Vaccinologists have demonstrated that we sometimes can thoughtfully tinker our way to useful vaccines— even when our understanding of the relevant biology is woefully incomplete. Like it or not, virtually every vaccine made to date was developed through considerable tinkering. But, thinking vs tinkering clearly

represents a false dichotomy. We need them both. As Donald Burke and his Walter Reed colleagues saliently observed years ago, "The history of vaccine development bears strong witness to the value of thoughtful empiricism" [49].

Contemporary understanding still does not permit highly reliable predictions regarding the efficacy of any candidate vaccine, and no known vaccine approach is guaranteed to work for HIV/AIDS-- or for any other novel pathogen, including SARS-CoV-2. All we know for sure is what has and has not worked in the past. But, given what is at stake, it surely is appropriate to consider the use of classical vaccinology and killed-virus vaccines whenever we confront pandemic disease. We do not know how well this strategy will operate for HIV, but there is no mystery about how to find out. All it would require is one group of dedicated vaccinologists and a single agency, philanthropy, or motivated individual to step up to the plate as when the March of Dimes supported the nationwide trial of Jonas Salk's killed-poliovirus vaccine in 1954. Until then, unfortunately, polio vaccines had been delayed for decades due to mistaken conclusions of able scientists-- all of them arguably acting with the best of intentions-- whose understanding of the relevant biology was incomplete-- and often completely wrong. So Salk's trial in American schoolchildren was run against the advice of most prominent biologists of that era. But his vaccine proved to be safe and effective [50]. Today, the killed-virus product is the only polio vaccine in routine use by pediatricians in the U.S and many other countries [51].

Nonetheless, given recent history and the unprecedented success of the SARS-CoV-2 mRNA vaccines for Covid-19 [52,53], isn't it reasonable to suppose that this new technology might be ready and able to replace classical methods like killed virus vaccines? It certainly is possible that mRNA encoding some version of the trimeric HIV envelope protein gp120 or gp160 would induce protection; but we don't yet know that. We do know that many challenges will confront HIV vaccines based on mRNA [54]. We might expect that any mRNA which encodes the native protein potentially will encounter the same fate as all other versions of the native HIV envelope tested to date-- which all have failed to elicit protective immunity. We do not know which alternative version might circumvent this issue. The only way to find out is to tinker. But we have no way to know whether our tinkering with this issue will require many years, or even many decades, or will ever succeed.

We are led to this compelling conclusion: If we start promptly and work with due urgency, and if killed-virus vaccines prove to be as effective for human immunodeficiency virus as they are for related animal retrovirus, it still may be possible to deploy safe, effective preventive AIDS vaccines by the year 2025. This effort would not be inexpensive, but it would pay for itself if it shortened the AIDS vaccine endeavor by three or four days. Were it to advance the end of AIDS by three or four decades, huge quantities of human and economic resources would be liberated for other uses. And we potentially might salvage reasonable lives for millions, or tens of millions, of men, women, and children who may fall victim to AIDS if we opt instead to wait for a vaccine from other more innovative and more interesting approaches.

So we ardently hope the proposals offered here will garner serious consideration and firm support from HIV/AIDS stakeholders, including the dedicated scientists striving to unravel the biological mysteries of human immunology and viral pathogenesis. Their work to date has produced great benefit and is certain to bear more fruit. But the aspiration to obtain better vaccines through basic science and the aspiration to extract any benefit available from classical empirical vaccinology are in no sense mutually exclusive. Both strategies deserve to be pursued with diligence and resolve. We do not get many chances to exert an impact this consequential, or to alter the course of history so dramatically. If we are to solve this problem just as soon as humanly possible, we arguably will need the diligent effort of both thinkers and tinkerers.

Acknowledgements

The author owes sincere thanks for helpful conversations over three plus decades with Drs. Michael Ascher, David Baltimore, Michael Botchan, Carlos Bustamante, Marcus Conant, Richard Creagan, Donald Francis, Richard Giles, Donald Kennedy, Jay Levy, John Oxford, Marc Van Regenmortel, Bruce Walker, Robin Weiss, and Bruce Weniger, for the advice and selfless efforts of Sam Dorman and Haynes Sheppard, and for the support and forbearance of other family members, most especially Ruth, as well as Jennie, Anna, and Jacob. But no one mentioned above should be presumed to concur with a single idea put forth in this essay.

References

1. Experimental HIV Vaccine Regimen Ineffective in Preventing HIV; No Safety Concerns Found; NIH and Partners Discontinue Vaccinations, USA.

2. Cohen J (2020) Another HIV vaccine strategy fails in large-scale study. Science 367: 611-2.

3. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al (2009). Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 361: 2209-20.

4. Boffey PM (April 29, 1984) A likely cause but still no cure. NY Times, USA.

5. Fields BN (1994) AIDS: time to turn to basic science. Nature 369: 95-6.

6. Douek DC, Kwong PD, Nabel GJ (2006) The Rational design of an AIDS vaccine. Cell 124: 677-81.

7. Nabel GJ (2012) Rational Design of Vaccines for AIDS and Influenza. Trans Am Clin Climatol Assn 123: 9-16.

8. Fauci AS (2009) Why there is no AIDS vaccine, USA.

9. Virgin HW, Walker BD (2010) Immunology and the elusive AIDS vaccine. Nature 464: 224-31.

10. Subbaraman H, Schanz M, Trkola A (2018) Broadly Neutralizing Antibodies: What is needed to move from a rare event in HIV-1 infection to vaccine efficacy? Retrovirology 15: 52-65.

11. Kim JH, Excler J-L, Michael NL (2015) Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. Ann Rev Med 66: 1-15.

12. Horwitz1 JA, Bar-On Y, Lu C-L, et al (2017) Non-neutralizing antibodies alter the course of HIV-1 infection in Vivo. Cell 170: 637-48.

13. Brett-Major DM, Crowell TA, Michael NL (2017) Prospecting for an HIV vaccine. Trop Dis Trav Med Vacc 3: 6.

14. Polara J, Easterhoff D, Fouda GG (2017) Lessons learned from HIV vaccine trials. Current Opinion 12: 216-21.

15. Lewis GK, Pazgier M, DeVico AL (2017) Survivors remorse: antibody-mediated protection against HIV-1. Immun Rev 275: 271-84.

16. Ackerman ME, Barouch DH, Alter G (2017) Systems Serology for evaluation of HIV vaccine trials. Immunol Rev 275: 262-70.

17. Haynes BF, Mascola JR (2017) The quest for an antibody-based HIV vaccine. Immun Rev 275: 5-10.

18. Hsu DC, O'Connell RJ (2017) Progress in HIV vaccine development. Hum Vaccines Immunother 13: 1018-30.

19. Van Regenmortel MHV (2018) Development of a Preventive HIV Vaccine Requires Solving Inverse Problems Which Is Unattainable by Rational Vaccine Design. Front Immun 8: 10.3389/fimmu.2017.02009.

20. Ackerman M, Alter G (2013) Mapping the journey to an HIV vaccine. NEJM 369: 389-91.

21. Hilleman MR (1992) Impediments, imponderables and alternatives in the attempt to develop an effective vaccine against AIDS. Vaccine 10:1053-8.

22. Clements-Mann ML (1998) Lessons for AIDS Vaccine Development from Non-AIDS Vaccines. AIDS Res Hum Retr 14: S197–S203.

23. Barouch DH (2013) The Quest for an HIV-1 Vaccine - Moving Forward. NEJM 369: 2073-6.

24. Hilleman MR (1998) A simplified vaccinologists' vaccinology and the pursuit of a vaccine against AIDS. Vaccine 16: 778-93.

25. Shedlock DJ, Silvestri G, Weiner DB (2009) Monkeying around with HIV vaccines: using rhesus macaques to define 'gatekeepers' for clinical trials. Nat Rev Immunol 9: 717-28.

26. Sheppard HW, Dorman BP (2015) Time for a systematic look at inactivated HIV vaccines. AIDS 29: 125-7.

27. Lutus, PA (Date unknown) Summary - key elements of problem-solving, USA.

28. Race E, Stein CA, Wigg MD (1995) A Multistep procedure for the chemical inactivation of human immunodeficiency virus for use as an experimental vaccine. Vaccine 13: 1567-75.

29. Sheppard HW (2005) Inactivated- or killed-virus HIV/AIDS vaccines. Curr Drug Targets Infect Disord 5: 131-41.

30. Issel CJ, Horohov DW, Lea DF et al (1992) Efficacy of inactivated whole-virus and subunit vaccines in preventing infection and disease caused by equine infectious anemia virus. J Virol 66: 3398-408.

31. Cranage MP, Polyanskaya N, McBride B (1993) Studies on the specificity of the vaccine effect elicited by nactivated simian immunodeficiency virus. AIDS Res Hum Retroviruses 9: 13-22.

32. Hosie MJ, Beatty JA (2007) Vaccine Protection against feline immunodeficiency virus: setting the challenge. Aust Vet J 85: 5-12.

33. Uhl EW, Martin M, Coleman JK, Yamamoto JK (2008) Advances in FIV vaccine technology. Vet Immunol Immunopathol 123: 65-80.

34. Medlock J, Panday A, Parpia A, Tang A, Skrip LA, et al. (2017) Effectiveness of UNAIDS targets and HIV vaccination across 127 countries. Proc Natl Acad Sci USA 114: 4017-22.

35. Lin G, Nara PL (2007) Designing immunogens to elicit broadly neutralizing antibodies to the HIV-1 envelope glycoprotein. Curr HIV Res 5: 514-41.

36. Burton DR, Ahmed R, Barouch DH (2012) A blueprint for HIV vaccine discovery. Cell Host & Microbe 12: 396-407.

37. Johnson PR, Montefiori DC, Goldstein S (1992) Inactivated whole-virus vaccine derived from a proviral DNA clone of simian immunodeficiency virus induces high levels of neutralizing antibodies and confers protection against heterologous challenge. Proc Natl Acad Sci USA 89: 2175-9.

38. Rossio JL, Esser MT, Suryanarayana K (1998) Inactivation of human immunodeficiency virus type 1 infectivity with preservation of conformational and functional integrity of virion surface proteins. J Virol 72: 7992-8001.

39. U.S. National Library of Medicine (2017) A Study to Assess the Efficacy of a Heterologous Prime/Boost Vaccine Regimen of Ad26.Mos4.HIV and Aluminum Phosphate-Adjuvanted Clade C gp140 in Preventing Human Immunodeficiency Virus (HIV) -1 Infection in Women in Sub-Saharan Africa (HVTN 705) Clinical trials.gov, USA.

40. U.S. National Library of Medicine (2019) A Study of Heterologous Vaccine Regimen of Adenovirus Serotype 26 Mosaic4 Human Immunodeficiency Virus(Ad26.Mos4.HIV), Adjuvanted Clade C gp140 and Mosaic gp140 to Prevent HIV-1 Infection Among Cis-gender Men and Transgender Individuals Who Have Sex With Cis-gender Men and/or Transgender Individuals (MOSAICO). Clinical trials.gov, USA.

41. Norrby E, Penttinen K (1978) Differences in antibodies to the surface components of mumps virus after immunization with formalin-inactivated and live virus vaccines. J Infec Dis 138: 672-6.

42. Blackburn NK, Besselaar TG (1991) A study of the effect of chemical inactivants on the epitopes of Rift Valley Fever virus glycoproteins using monoclonal antibodies. J Virol Meths 33: 367-74.

43. Thalhamer J, Freund J. (1985) Passive immunization: a method of enhancing the immune response against antigen mixtures, J Immunol Meth 80: 7-13.

44. Sattentau Q. (1995) Conservation of HIV-1 gp120 neutralizing epitopes after formalin inactivation. AIDS 9: 1383-5.

45. Nathanson N, Langmuir AD (1963) The Cutter Incident: Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States during the spring of 1955: I. Backgound. Am J Hyg 78: 16-28.

46. Langmuir AD, Nathanson N, Jackson Hall W (Oct. 13, 1955) The Wyeth Problem: an epidemiological analysis of the occurrence of poliomyelitis in association with certain lots of Wyeth vaccine, USA.

47. Sheets RL, Goldenthal KL (1998) Traditional approach to preventive HIV vaccines: What are the cell substrate and inactivation issues? AIDS Res Hum Retroviruses 14: 627-33.

48. Shultz AM, Koff WC, Lawrence DN (8 February 1990) Workshop on HIV Inactivated Vaccines, Bethesda, Maryland, USA.

49. Mascola JR, McNeil JG, Burke DS (1994) AIDS Vaccines. Are we ready for human efficacy trials? JAMA 272: 488-9.

50. Bodian Bodian (1976) Poliomyelitis and the Sources of Useful Knowledge. Johns Hopkins Med J 138: 130-6.

51. American Academy of Pediatrics (2019) Immunization Schedules for 2019, USA.

52. Polack FP, Thomas SJ, Kitchen N (2020) Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. NEJM 383: 2603-15.

53. Baden LR, El Shaly HM, Essink B (2021) Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. NEJM 384: 403-16.

54. Mu Z, Haynes BF, Cain DW (2021) HIV mRNA Vaccines--Progress and Future paths. Vaccines 9: 134.