AIDS Vaccines and Preexposure Prophylaxis: Is Synergy Possible?

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Abstract

While the long-term goal is to develop highly effective AIDS vaccines, first generation vaccines may be only partially effective. Other HIV prevention modalities such as preexposure prophylaxis with antiretrovirals (PrEP) may have limited efficacy as well. The combined administration of vaccine and PrEP (VAXPREP), however, may have a synergistic effect leading to an overall benefit that is greater than the sum of the individual effects. We propose two test-of-concept trial designs for an AIDS vaccine plus oral or topical ARV. In one design, evidence that PrEP reduces the risk of HIV acquisition is assumed to justify offering it to all participants. A two-arm study comparing PrEP alone to VAXPREP is proposed in which 30 to 60 incident infections are observed to assess the additional benefit of vaccination on risk of infection and setpoint viral load. The demonstrated superiority of VAXPREP does not imply vaccine alone is efficacious. Similarly, the lack of superiority does not imply vaccine alone is ineffective, as antagonism could exist between vaccine and PrEP. In the other design, PrEP is assumed not to be in general use. A 2×2 factorial design is proposed in which high-risk individuals are randomized to one of four arms: placebo vaccine given with placebo PrEP, placebo vaccine given with PrEP, vaccine given with placebo PrEP, or VAXPREP. Between 60 and 210 infections are required to detect a benefit of vaccination with or without PrEP on risk of HIV acquisition or setpoint viral load, with fewer infections needed when synergy is present.

Introduction

UNAIDS ESTIMATES THAT in 2008 2.7 million people worldwide became newly infected with HIV and 2 million people died from AIDS. The total number of people living with HIV is estimated to be near 33.4 million, with 97% living in low- and mid-income countries and 48% being women.¹ Preventing HIV infection and AIDS is a global priority, and a number of behavioral, barrier, antiretroviral drug-based and vaccine approaches have been the subject of intense research.^{2,3} While antiretroviral treatment (ART) in HIV-infected individuals slows progression to AIDS and death, its benefit at a population level is dependent on the availability of drugs and adherence to treatment regimens. A safe, effective, and durable vaccine is needed to stem the pandemic.

The development of an AIDS vaccine has faced many challenges over the past 25 years. Current AIDS vaccine candidates do not elicit broadly neutralizing antibodies capable of preventing infection by the vast diversity of HIV isolates.⁴ The first test-of-concept trial of a cell-mediated immunity (CMI)-based vaccine did not show efficacy and raised the concern that the vaccine might enhance the risk of infection for some vaccinees.⁵ Encouragingly, the recent RV144 Thai trial of a prime-boost vaccine regimen aiming at inducing both CMI and antibody responses showed in a modified intention-to-treat analysis a 31% reduction in the risk of infection.⁶ Vaccine candidates in current trials are designed to induce HIV-specific CMI responses that could slow HIV replication, destroy HIV-infected cells, preserve immune memory, delay disease progression, and possibly prevent

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secondary transmission by reducing the quantity of virus in body fluids (viral load).⁷⁻⁹

Other HIV prevention modalities have shown promise. Prompt postexposure prophylaxis reduces the risk of transmission due to occupational¹⁰ or sexual exposure.^{11,12} Treatment of mother and/or child before and after childbirth has reduced the risk of mother-to-child transmission.^{13,14} Very recently, the first successful demonstration of protection by tenofovir-containing vaginal gel has provided proof-of-concept for topical microbicide preexposure prophylaxis (PrEP). Tenofovir gel reduced HIV acquisition by an estimated 39% overall, and by 54% in women with high adherence to the dosing regimen of before and after sexual exposure.¹⁵ Animal studies have provided evidence that PrEP and postexposure prophylaxis (PEP) with tenofovir disoproxil fumarate (TDF), alone or in combination with emtricitabine (FTC), can protect against simian immunodeficiency virus (SIV) and chimeric simian HIV (SHIV) infection.¹⁶⁻²² Furthermore, in some experiments, animals that became infected despite receiving TDF as PrEP or PEP showed delayed onset of viremia and delayed seroconversion,^{18,20,23} demonstrating that even in the case of incomplete protection against infection, PrEP or PEP may lead to attenuated acute infection (perhaps by allowing the immune system to gain some control of the virus). The demonstrated efficacy of TDF in the treatment of HIV infection, the lack of reported serious safety problems associated with its use,²⁴ its long half-life,²⁵⁻²⁸ and its relatively high threshold to drug resistance²⁹ make this antiretroviral agent an attractive candidate for systemic or topical prophylactic use. Tenofovir and related drugs have high concentrations in genital tissues,³⁰⁻³² so repeated exposure with locally aborted HIV infection is theoretically possible and, if it occurs, may lead to the development of HIV-specific cellular immune responses. Several clinical efficacy trials using TDF alone or in combination for oral or topical prophylaxis are ongoing (www.prepwatch.org). Other drugs are also being considered for use in such regimens.³³ However, more safety and efficacy data are needed, and there are significant practical barriers to widespread prophylactic use of ARV.³⁴

ART for HIV-infected individuals may prove useful in the prevention of secondary transmission. Viral load is the single greatest risk factor for all modes of transmission.^{35,36} Sexual transmission of HIV has been closely linked to viral load in the blood of the infected host,³⁷⁻⁴¹ which probably serves as a surrogate, albeit imperfect, for HIV concentration in the genital tract.^{42,43} In addition to lowering plasma viral load to nearly undetectable levels, ART can decrease viral load in genital secretions,^{44,45} although patients having a detectable semen HIV load may have no detectable virus in their blood plasma, highlighting the residual risk of HIV-1 transmission during unprotected intercourse.⁴⁶ Observational studies and recent modeling work have triggered considerable interest and concerns regarding the use of ART in HIV-infected individuals to prevent secondary transmission.^{32,47-54}

In the coming years, systemic PrEP or drug-based microbicides (which in this discussion is considered a topical form of PrEP) may be proven to safely confer protection against HIV infection. In that instance, even though the implementation of a "background" intervention would require careful consideration by national governments,⁵⁵ PrEP might be proposed as a baseline intervention for AIDS vaccine trial

participants. In effect, by allowing repeated exposures to HIV that do not lead to established infection, PrEP may allow exposure-induced immunity to alter vaccine-induced immunity; on the other hand, vaccination could influence susceptibility, thus altering the efficacy of PrEP. Similarly, ART in HIV-infected individuals to prevent secondary transmission may gain momentum in populations with a high prevalence of HIV infection. Low-dose exposure, presumably more common when the donor is receiving ART, which can reduce virus concentration in both blood and genital secretions, might therefore be countered more easily by preexisting, vaccine-induced immunity in the recipient. These possibilities offer new opportunities for combination biomedical HIV prevention research and raise several questions about potential interactions and/or synergies between PrEP, ART for prevention, and AIDS vaccines. Such potential interactions should be considered when designing intervention trials and when "translating" the results of randomized, controlled trials to predictions of how the interventions, when introduced into broad use, may influence public health.

Hypotheses

When given in combination, an AIDS vaccine and PrEP could act synergistically to reduce the risk of HIV infection or attenuate the initial infection, thereby slowing disease progression. The overall benefit of the combination could be greater than the sum of the individual benefits of vaccine and PrEP. In this article we describe evidence in animals and humans to support this notion and examine the design of clinical trials exploring simultaneous use of an AIDS vaccine and PrEP. In this section, evidence is presented to support two hypotheses:

- PrEP may reduce the infectious exposure sufficiently that a person can withstand it, especially if she or he has preexisting, vaccine-induced immunity.
- Repeated abortive infections (potentially possible under PrEP) may allow mucosal or systemic immunization that could be enhanced by vaccine priming and/or boosting.

Weaker challenge dose favors vaccine protection

In the SIV model, vaccines work better against lower virus concentration and low-dose repeated challenges.⁵⁶⁻⁵⁸ By reducing initial HIV viral replication, PrEP might convert a strong HIV "challenge" (through natural exposure) into a weaker challenge that a modest level of vaccine-induced immunity could overcome, preventing establishment of HIV infection or leading to a lower viral load and slower disease progression in the long term. Additionally, when given around the time of vaccination, PrEP might provide a protective "cover" if there is a transient period of enhanced susceptibility during and shortly after vaccination, followed by a period of immunity.

Mucosal exposure in the absence of chronic infection can induce immune responses

In some nonhuman primate (NHP) experiments, virus-specific mucosal immune responses have been detected after a single low-dose exposure to SIV by the rectal route in the absence of overt infection.⁵⁹ Both timing and location may be

critical determinants of outcome prevention in the earliest stages of infection. When infected founder populations are small and focal, and have not as yet expanded sufficiently to disseminate and establish systemic infection, a relatively small number of effectors at the mucosal site of entry might be at the right place, "enough and soon enough" to clear infection,⁶⁰⁻⁶² and this aborted infection might induce immune responses.

Animal experiments suggest that sequential mucosal and systemic (or reverse order) vaccinations can contribute to a better immune response to mucosal challenges.⁶³⁻⁶⁵ Systemic vaccination followed by continued mucosal exposures in the presence of PrEP that is sufficient to prevent established infection may allow more effective development of mucosal response. The possibility of a detrimental interaction between ARV for prevention and vaccination has not yet been assessed.

The prognostic significance of HIV-specific cytotoxic T lymphocyte (CTL) responses in highly exposed seronegative individuals is unknown. A systematic review of these studies suggests that natural resistance to HIV infection in highly exposed individuals is mediated by multiple mechanisms, conferring on them an "immunologic advantage" that may be related to innate and/or adaptive immune systems.^{66,67} Although offering only a partial explanation to the natural resistance to HIV infection,⁶⁸ observations in female sex workers in Nairobi with frequent HIV exposure,⁶⁹⁻⁷¹ in exposed uninfected infants with strong HIV-1-specific T cell responses,⁷² and more recently in discordant couples⁷³ suggest that recurrent viral exposure may lead to priming of HIV-specific systemic and mucosal immune responses. These findings suggest that persons receiving PrEP who are exposed to HIV may generate HIV-specific T cell responses, potentially contributing to protection against the establishment of HIV infection. These immune responses could possibly boost or prime vaccine-induced responses.

One scenario for vaccine and PrEP synergy would include a short period of limited mucosal infection terminated by PrEP, which would boost existing vaccine-induced responses. In this sense, PrEP would allow mucosal "vaccination," which has been shown to boost virus-specific immune responses in animal studies. Systemic prime followed by mucosal boost vaccination or mucosal prime followed by systemic boost can lead to stronger systemic responses compared to either separately, although for the systemic-mucosal sequence both groups exhibited equivalent, significant protection and robust post-SIV challenge cellular immunity.^{30,61-63}

PrEP may lead to abortive or controlled infection associated with immune responses

Experiments in macaques support the idea that anti-retroviral drugs given around the time of infection can suppress viral replication in infected animals sufficiently to allow the development of immune responses.⁷⁴ Mathematical modeling of ARV treatment during acute SIV infection suggests that transient treatment minimizes immune impairment and allows immune sensitization, leading to high levels of memory CTL for long-term SIV control.⁷⁵

SIV exposure followed by PrEP may activate immune responses to protect against subsequent infection with heterologous SIV challenge virus.^{17,76} Transient early treatment

with tenofovir in macaques infected with SIV⁷⁶⁻⁸¹ and SHIV⁸² has been associated with the stimulation of SIV- or SHIV-specific CD4⁺ and CD8⁺ T cell responses and resistance to rechallenge with heterologous virus more than 1 year following the initial challenge.⁸³⁻⁸⁵ A possible interpretation of these results is that drug treatment suppressed viral replication in the infected animals sufficiently to allow development of effective immune responses that controlled or eliminated SIV.^{23,75,86,87} However, in other studies, animals did not develop readily measurable cellular anti-SIV immune responses, and did not resist homologous rechallenge with SIVmac239.⁸⁸

Rhesus macaques experimentally infected with SHIV while on ART showed long-lasting virus-specific cell-mediated immune responses despite low and/or undetectable viral loads compared to control animals, leading to long-term disease protection.⁸⁹ The generation of immune responses in PrEP-infected animals could lead to synergy with vaccine-induced immune responses. Nonhuman primate studies support the concept of a significant protective effect against repeated low dose intrarectal SIV and SHIV challenge, after rectal application of tenofovir prior to virus challenge.²⁰ The hypothesis is that extremely low levels of virus replication could occur during PrEP and induce virus-specific immune responses. Macaques protected by PrEP from challenge may harbor occult virus that may be initially contained by cytotoxic T cells, but could emerge later if immunity fades. Interestingly, in the absence of seroconversion, gag-specific interferon (IFN)- γ -secreting T cells were detected in the blood of protected animals.⁷⁴ Other studies using CD8 depletion showed that there was no virus present, suggesting that PrEP may have cleared the virus.^{90,91}

In humans, although anecdotal, drug-susceptible HIV-1 infection despite intermittent fixed-dose combination TDF/FTC as prophylaxis was associated with low-level viremia, delayed seroconversion, attenuated clinical course, and relative sparing of mucosal CD4⁺ T cells in the gastrointestinal tract of a patient. Although HIV-specific immune responses were not collected, this observation suggests an associated immune mechanism.⁹² More immunological data are expected from ongoing studies.⁵⁵

Clinical Trial Designs

Nonhuman primate studies could test the concept of AIDS vaccine and PrEP synergy to protect against HIV infection or to modulate disease.^{93,94} This article focuses exclusively on clinical trial designs in humans. Two clinical trial designs are proposed.

Two-arm trial

The first design assumes that PrEP efficacy is firmly established in human trials; it aims at detecting additional benefit from an AIDS vaccine candidate. The basic AIDS vaccine and PrEP (VAXPREP) trial design is a two-arm, randomized, double-blind study comparing a single AIDS vaccine candidate to vaccine placebo in a setting in which all participants receive open-label PrEP. Entry criteria would be such that the expected HIV incidence rate would be at least 2% per year.

After a run-in period to assess compliance with PrEP, individuals who tolerate PrEP and take at least 80% of intended doses would be randomized in a 1:1 ratio to receive either the

AIDS vaccine candidate or placebo. The optimal dose and schedule for the vaccine would be based on data from previous Phase I/II trials. Vaccination is assumed to take 6 months after which participants would be tested for HIV infection every 3 months. Participants would be followed for a minimum of 12 months after vaccination is completed. PrEP would be given throughout the trial period, although a brief period of post-PrEP observation might be used at the end of the trial to ensure that there were no infections masked by PrEP or vaccine.

HIV infection would be determined by a prespecified diagnostic algorithm including a serological test that can distinguish between vaccination and HIV infection and nucleic acid detection assay. Trial participants who become HIV infected would discontinue PrEP and have their HIV RNA plasma viral load measured at two time points postinfection, approximately 3–6 months after PrEP is stopped, with the geometric mean of the measurements used as their setpoint viral load. The time points correspond to at least 3 and 6 months after the initial positive HIV test, which would exclude acute HIV infection viral load peaks. Infected participants would be offered enrollment into a long-term follow-up study to assess trends in postinfection outcomes such as disease progression, viral load, CD4⁺ T cell count, and drug resistance.

Trial participants found to be HIV infected at the time of the last dose of study vaccine or placebo would have baseline specimens assessed by PCR to rule out a preexisting infection. Those found to be infected at baseline would be excluded from the modified intention-to-treat (mITT) analysis set for the principal evaluation of VAXPREP efficacy. These participants, however, would be included in the safety evaluation of VAXPREP.

We postulate that the combination of an AIDS vaccine and PrEP may reduce the risk of HIV infection or reduce the plasma viral load in those who become infected, relative to PrEP alone. In statistical terms, we treat HIV infection and viral load as coprimary endpoints and test a composite null hypothesis that the vaccine neither reduces the risk of HIV infection nor the viral load at setpoint. The composite null hypothesis is expressed as H_0 : $VEs = 0$ and $\Delta VL = 0$, where VEs is the percent reduction in the cumulative HIV incidence due to vaccination and ΔVL is the difference in average viral load measured on a $\log_{10}$ scale between vaccine recipients and placebo recipients who become infected. Following the approach of the Step trial,⁹⁵ we would test H_0 against the one-sided alternative H_1 : $VEs > 0$ or $\Delta VL > 0$. (See the Appendix for detailed statistical considerations.)

Table 1 presents the total number of incident HIV infections required to have 80% power to reject the composite null hypothesis for different values of VEs and ΔVL . In these calculations, we have assumed that PrEP by itself reduces the risk of HIV acquisition by 50% in the face of adherence similar to that seen in PrEP trials and reduces setpoint viral load from an average of 4.5 to 4.0 $\log_{10}$. Individual setpoints are assumed to vary with a standard deviation of 1.0 $\log_{10}$, a value somewhat larger than that in cohorts such as the MACS,⁹⁵ but in line with at-risk groups in Africa (IAVI, unpublished data). We also assume no postrandomization selection bias for viral load. For example, if vaccination was more likely to prevent infection in vaccinees who would have had higher viral loads had they not been vaccinated, then viral load might appear

TABLE 1. TOTAL NUMBER OF INCIDENT HIV INFECTIONS REQUIRED TO HAVE 80% POWER TO REJECT THE COMPOSITE NULL HYPOTHESIS H_0 : $VEs = 0$ AND $\Delta VL = 0$ FOR A 2-ARM VAXPREP VS. PREP TRIAL

VEs^a	ΔVL			
	0.25 $\log_{10}$	0.5 $\log_{10}$	0.75 $\log_{10}$	1.0 $\log_{10}$
0%	>100	>100	66	44
10%	>100	>100	58	36
20%	>100	98	53	33
30%	>100	75	45	<30
40%	87	55	36	<30
50%	55	40	<30	<30
60%	36	<30	<30	<30

Based on a one-sided, 0.05 level Fisher test. VAXPREP, vaccine plus PrEP. PREP, vaccine placebo plus PrEP.

^a $VEs = 1 - \text{cumulative HIV incidence (VAXPREP)} / \text{cumulative HIV incidence (PREP)}$.

artificially lower in HIV-infected vaccinees. Study power would be reduced if the analysis were adjusted for such selection bias. This form of selection bias, however, is unlikely.

This trial would be designed to have at least 80% power to detect benefit of the VAXPREP combination, compared to PrEP alone, when VEs is at least 30% and setpoint viral load is further reduced by at least 1.0 $\log_{10}$. To detect such an effect, we require 30 total incident HIV infections. If 90% power is desired in a VAXPREP trial, about 45 total infections would be required. If VAXPREP is unlikely to have an effect on setpoint viral load, then the trial would be designed to detect a 50% reduction in risk of HIV infection for which about 60 total infections are needed to have 80% power.

Table 2 displays the required sample size for a VAXPREP trial in which 30 HIV infections are expected assuming various background rates of HIV infection and lengths of follow-up. Sample size calculations assume 90% of participants will adhere to PrEP during the run-in period and will subsequently be randomized. Enrollment is expected to be completed in 1 year. A 10% loss to follow-up rate during the 6-month vaccination period and a 10% annual loss to follow-up rate during the postvaccination follow-up period are assumed. We recommend enrolling a sufficient number of participants so that 24 months of follow-up would suffice to complete the trial in 3 years. Thus, from 900 to 2500 high-risk individuals would be enrolled over a period of approximately 12 months.

Given the small number of events required to assess efficacy, the trial would not have a planned interim efficacy analysis. However, the safety of trial participants would be assessed on an ongoing basis. To rule out enhancement of HIV infection by the vaccine regimen, a special interim safety analysis could be conducted after 20 HIV infections are observed or on a more frequent schedule as deemed appropriate.

This two-arm design cannot determine whether a synergistic effect exists between vaccine and PrEP since there is no group receiving vaccine alone. It is theoretically possible that vaccine and PrEP have a synergistic effect when given in combination such that the benefits of VAXPREP exceed those of PrEP while there is little or no benefit when vaccine

TABLE 2. SAMPLE SIZE REQUIRED TO ACHIEVE 30 INCIDENT HIV INFECTIONS IN A 2-ARM VAXPREP (VACCINE PLUS PrEP) TRIAL FOR DIFFERENT HIV INCIDENCE RATES, VACCINE EFFICACY (VEs) AND FOLLOW-UP PERIODS

VEs	Annual HIV incidence	Follow-up period		
	Under PrEP	18 months	24 months	30 months
0%	1.0%	2697	2086	1720
	1.5%	1891	1465	1209
	2.0%	1353	1050	869
	2.5%	1085	844	699
10%	1.0%	2837	2195	1809
	1.5%	1989	1541	1271
	2.0%	1423	1104	913
	2.5%	1141	887	734
20%	1.0%	2993	2315	1908
	1.5%	2098	1625	1341
	2.0%	1500	1164	962
	2.5%	1203	935	774
30%	1.0%	3168	2450	2019
	1.5%	2220	1719	1418
	2.0%	1588	1231	1017
	2.5%	1272	988	818
40%	1.0%	3365	2602	2144
	1.5%	2358	1826	1506
	2.0%	1686	1307	1080
	2.5%	1351	1049	868

Ninety percent of participants are assumed to tolerate and comply with PrEP during a 1-month run-in period and are subsequently randomized. PrEP alone is assumed to reduce risk of HIV infection by 50%. A 10% loss to follow-up rate during the 6-month vaccination period and a 10% annual loss to follow-up rate during postvaccination follow-up are assumed. Effect of vaccination is assumed to start after first dose of the vaccine. All incident HIV infections are counted from randomization.

is administered alone. Thus, we could not infer that the vaccine alone is beneficial from a two-arm VAXPREP trial. It is also possible that vaccine and PrEP have an antagonistic effect such that VAXPREP is no better than PrEP while the vaccine alone is as efficacious as PrEP and with perhaps a better safety profile. An efficacious vaccine with a two-arm design could potentially be missed. Preclinical and immunogenicity studies using a factorial design might rule out these scenarios. However, careful consideration of these limitations of a two-arm design is needed when contemplating such a trial.

Two-by-two factorial trial

The second trial design assumes PrEP is not in general use and utilizes a 2×2 factorial design. After a 1- or 2-month run-in period to assess compliance with placebo PrEP, participants who take at least 80% of intended doses would be randomized to one of four study arms: vaccine plus PrEP (VAXPREP), placebo vaccine plus PrEP (PREP), vaccine plus placebo PrEP (VACCINE), and placebo vaccine plus placebo PrEP (PLACEBO). PrEP or its corresponding placebo would be given throughout the trial period.

The primary objective of the trial is to assess the effect of vaccination with or without the use of PrEP on the risk of HIV

acquisition and setpoint viral load. An important secondary objective is to determine the effect of PrEP without vaccination on risk of infection and viral load. Should VACCINE or VAXPREP be shown to be superior to PLACEBO, it is important to know whether the effect of VACCINE or VAXPREP is greater than the effect of PREP.

To assess the so-called "main" effect of vaccination, we would calculate the relative change in HIV incidence (VEs) and the difference in average setpoint viral load (AVL) between the VACCINE and PLACEBO groups. Likewise, to assess the main effect of PrEP, we would compare outcomes between the PREP and PLACEBO groups. To assess the overall benefit of the combination of vaccine and PrEP, we would compare outcomes between the VAXPREP and PLACEBO groups. We would recommend the vaccine with or without PrEP for further testing if either vaccine alone or vaccine in combination with PrEP demonstrates a positive benefit at an appropriate significance level. (See the Appendix for detailed statistical considerations.)

Table 3 illustrates the potential main effects of vaccination as well as the overall effect of vaccination with PrEP. We assume that PrEP alone reduces the risk of infection by 30%, but has no effect on setpoint viral load. Scenario A highlights the situation in which the main effect of vaccination is to reduce setpoint viral load by 1.0 log₁₀ without changing the risk of HIV infection. Assuming the effects of vaccination and PrEP are additive, we expect a 30% reduction in the risk of HIV infection and a 1.0 log₁₀ reduction in setpoint viral load when we compare VAXPREP to PLACEBO. If the effects are synergistic, we expect a >30% reduction in risk of infection and/or a >1.0 log₁₀ reduction in setpoint. In our example of synergy, there is a 50% overall reduction in the risk of infection due to VAXPREP and a 1.0 log₁₀ further reduction in viral load. In Scenario B, we have a vaccine that reduces the risk of infection by 30%, but has no effect on setpoint viral load. This scenario is similar to what was observed in the RV144 vaccine trial.⁶ If the effects of vaccine and PrEP are additive, we expect an overall reduction of 51% in the risk of HIV acquisition, but no effect on setpoint viral load. Additivity is defined by a stage model⁹⁶ in which PrEP is assumed to be the first line of defense against HIV acquisition. If this line is breached, vaccine-induced immune responses become the second line of defense. Each stage is assumed to function independently of the other. Thus, in a population with a 2% annual rate of HIV infection, PrEP is expected to reduce this rate by 30% to 1.4%. Vaccine-induced responses then reduce the 1.4% acquisition rate after PrEP by 30% to 0.98%. On the other hand, if the effects are synergistic, then we expect a >51% reduction in risk. In our example, the net effect of VAXPREP is to reduce the risk of infection by 67%. Scenario C depicts a vaccine that reduces the risk of infection by 30% and reduces setpoint viral load by 1.0 log₁₀ when administered alone. Assuming additive effects, we expect a 51% reduction in the risk of infection and a 1.0 log₁₀ reduction in setpoint when we compare VAXPREP to PLACEBO. Greater reductions in risk of infection or setpoint would suggest synergy. Finally, in Scenario D, we consider a vaccine that actually enhances the risk of infection by 30%, but has no effect on setpoint viral load. If the effects of vaccine and PrEP are additive, we expect only a 9% reduction in risk of infection when we compare VAXPREP to PLACEBO. Furthermore, PREP outperforms VAXPREP in this situation.

TABLE 3. HYPOTHETICAL SCENARIOS FOR THE EFFECTS OF VACCINE, PREP, AND VAXPREP ON RISK OF HIV INFECTION AND SETPOINT VIRAL LOAD (VL, $\log_{10}$) FOR A 2×2 FACTORIAL TRIAL

	PLACEBO	PREP	VACCINE	VAXPREP	
				Additive	Synergistic
Scenario A ^a					
HIV incidence	2.0%	1.4%	2.0%	1.4%	1.0%
Setpoint VL	4.5	4.5	3.5	3.5	2.5
Scenario B ^b					
HIV incidence	2.0%	1.4%	1.4%	0.98%	0.67%
Setpoint VL	4.5	4.5	4.5	4.5	4.5
Scenario C ^c					
HIV incidence	2.0%	1.4%	1.4%	0.98%	0.67%
Setpoint VL	4.5	4.5	3.5	3.5	2.5
Scenario D ^d					
HIV incidence	2.0%	1.4%	2.6%	1.82%	1.0%
Setpoint VL	4.5	4.5	4.5	4.5	4.5

^aScenario A: the vaccine alone reduces setpoint viral load by 1.0 $\log_{10}$ without effect on HIV acquisition.

^bScenario B: the vaccine reduces HIV acquisition by 30% without effect on setpoint viral load.

^cScenario C: the vaccine reduces HIV acquisition by 30% and setpoint viral load by 1.0 $\log_{10}$.

^dScenario D: the vaccine enhances HIV acquisition by 30%, but has no effect on setpoint viral load.

PLACEBO, vaccine placebo and PrEP placebo; PREP, vaccine placebo and PrEP; VACCINE, PrEP placebo and vaccine; VAXPREP, vaccine and PrEP.

Table 4 shows the required number of incident HIV infections to have 80% or 90% power to detect a benefit of vaccination with or without PrEP under the scenarios described in Table 3. A randomization ratio of 1:1:1:1 across the four study arms is assumed. If the candidate vaccine is expected to reduce the setpoint viral load by at least 1.0 $\log_{10}$ without decreasing the risk of HIV infection appreciably, then a factorial design with about 60 total incident infections will be adequate to detect such a benefit of vaccination. Such is the case with Scenarios A and C. On the other hand, if the candidate is not expected to affect the setpoint, but may reduce the risk of infection with or without PrEP (Scenario B), then 80 to 210 total events are required depending on the desired study

power and the prior expectation for possible synergy. If pre-clinical studies, for example, support the possibility of synergy, then a factorial design with as few as 80 events will be sufficient to detect a benefit of vaccination when VAXPREP reduces the risk of infection by at least 67%. If VAXPREP is expected to reduce the risk of infection by 50%, then at least 160 infections are required for a trial with 80% power.

Scenario D poses some complex challenges. If the effects of vaccine and PrEP were additive, then the factorial design would be similar in size to a Phase III efficacy trial. However, we are not likely to design a trial to detect such effects. Furthermore, with interim monitoring for safety, the enhancement of HIV infection by vaccine alone might be detected and require the termination of enrollment if enrollment is not already complete.

As a test-of-concept trial, the factorial design will not have adequate power to demonstrate the superiority of VAXPREP to VACCINE or of VAXPREP to PREP unless there is a very large synergistic effect. However, the factorial design may have reasonable power to rank the benefits of PREP, VACCINE, and VAXPREP. Even if the effects of vaccine and PrEP are additive, their combination may be a useful prevention modality.

As an illustration of the size of a 2×2 factorial trial, Table 5 gives the required sample size to achieve 80 total incident infections. Assuming 24 months of follow-up, we would need to enroll between 2400 and 4000 participants over a 12- to 18-month period.

TABLE 4. TOTAL NUMBER OF INCIDENT HIV INFECTIONS REQUIRED TO DETECT A BENEFIT OF VACCINE OR VAXPREP IN A 2×2 FACTORIAL TRIAL WITH 80% AND 90% POWER

	VAXPREP with additive effects		VAXPREP with synergistic effects	
	80%	90%	80%	90%
Scenario A ^a	59	78	28	34
Scenario B ^b	157	210	79	103
Scenario C ^c	42	56	24	28
Scenario D ^d	>300	>300	217	285

Based on 10,000 simulated trials using R 2.10.1.

^aScenario A: the vaccine alone reduces setpoint viral load by 1.0 $\log_{10}$ without effect on HIV acquisition.

^bScenario B: the vaccine reduces HIV acquisition by 30% without effect on setpoint viral load.

^cScenario C: the vaccine reduces HIV acquisition by 30% and setpoint viral load by 1.0 $\log_{10}$.

^dScenario D: the vaccine enhances HIV acquisition by 30%, but has no effect on setpoint viral load. This trial would likely be stopped early for futility.

Discussion

First generation AIDS vaccines may have limited efficacy due to immune responses that are insufficient to prevent infection from the naturally occurring inoculum of HIV found in genital secretions. The use of PrEP in combination with vaccine may lower the HIV inoculum to a level that vaccine-induced immune responses can either block or attenuate infection. PrEP

TABLE 5. SAMPLE SIZE REQUIRED TO ACHIEVE 80 INCIDENT HIV INFECTIONS IN A 2×2 FACTORIAL TRIAL OF VAXPREP FOR DIFFERENT HIV INCIDENCE RATES, ADDITIVE/SYNERGIC EFFECTS, AND FOLLOW-UP PERIODS

<i>Annual HIV incidence</i>				<i>Study follow-up</i>		
<i>PLACEBO^a</i>	<i>PREP^a</i>	<i>VACCINE^a</i>	<i>VAXPREP^a</i>	<i>18 months</i>	<i>24 months</i>	<i>30 months</i>
2.00%	1.40%	1.40%	0.98%	4650	3599	2967
2.00%	1.40%	1.40%	0.67%	4911	3801	3134
2.00%	1.40%	1.40%	0.50%	5068	3922	3234
3.00%	2.10%	2.10%	1.47%	3104	2406	1987
3.00%	2.10%	2.10%	1.00%	3279	2542	2100
3.00%	2.10%	2.10%	0.75%	3385	2625	2168

Ninety percent of participants are assumed to tolerate and comply with PrEP during the 6-month run-in period and are subsequently randomized. A 10% loss to follow-up rate during the 6-month vaccination period and a 10% annual loss to follow-up rate during postvaccination follow-up are assumed. Effect of vaccination is assumed to start after the first dose of vaccine. All incident infections are counted from randomization.

^aPLACEBO, vaccine placebo and PrEP placebo; PREP, vaccine placebo and PrEP; VACCINE, PrEP placebo and vaccine; VAXPREP, vaccine and PrEP.

may also permit the induction of HIV-specific immune responses that boost the vaccine responses. Thus, there may be a synergistic effect between vaccine and PrEP. Test-of-concept trials of VAXPREP can assess this potential for synergy.

We recommend the implementation of a 2×2 factorial design when PrEP is not recommended for general use. Our implementation of a factorial design, however, is not the typical usage.⁹⁷ The Physicians' Health Study, for example, used a factorial design to assess the marginal effect of aspirin on cardiovascular disease and to assess the marginal effect of beta-carotene on cancer.⁹⁸ For this study, it was reasonable to assume that aspirin had no effect on risk of cancer and beta-carotene had no effect on risk of cardiovascular disease. The study was highly efficient and resulted in "two studies for the price of one." In our setting, whether VACCINE is better than PLACEBO or VAXPREP better than PLACEBO is the key question. If VACCINE or VAXPREP is better than PLACEBO, we want some sense of whether the benefit is better than that of PREP. As a test-of-concept trial, our study is not designed to demonstrate a significant interaction between vaccine and PrEP. However, if there is a strong synergistic effect, then the observed reduction in HIV incidence or setpoint viral load due to VAXPREP may be appreciably greater than that observed due to VACCINE and to PREP, more so than that predicted by the assumption of additive effects. The combination of PrEP and vaccine could also perform worse than either alone. Such observations could guide further testing of VAXPREP.

If PrEP safety and efficacy are demonstrated in humans, and it is deemed of public health benefit, PrEP may be used as background intervention in other HIV prevention trials including those of preventive AIDS vaccines. In this situation, the two-arm VAXPREP design may be appropriate. However, several efficacy trials of PrEP may be needed in various regions and populations with different formulations before careful consideration of its general use can be made. If the vaccine and PrEP were truly synergistic, then a comparison of VAXPREP and PREP might not be a reliable guide to the value of the vaccine alone.

Widespread adoption of PrEP would also raise new regulatory and operational issues for AIDS vaccine trials, including the establishment of national and/or international guidelines for PrEP provision in prevention trials, while

dealing with the absence of such guidelines in some countries as well as with drug supply and distribution. These guidelines would encompass the development of new skills and study procedures covering adherence counseling and monitoring, safety monitoring (in particular tenofovir renal⁹⁹ and bone density toxicity,¹⁰⁰ if TDF is used, although unlikely to be monitored in resource-limited settings), and antiretroviral drug resistance monitoring.

The proposed clinical trial designs are faced with multiple challenges. From a methodological point of view, several difficulties arise: the need for a larger sample size, issues with PrEP adherence, and enrollment criteria. Unless trials are designed to focus on a decrease in setpoint viral load, VAXPREP trials will be significantly larger than previously proposed Screening Test of Concept (STOC) trials,¹⁰¹ which reinforces the need to conduct multicenter and multicountry trials to meet sample size requirements. However, some consideration for restricting inclusion criteria is needed to limit the heterogeneity of participants enrolled. Ideally, a test-of-concept trial should enroll as homogeneous a population as possible to maximize statistical power.¹⁰² Despite aiming for homogeneity, in some settings both men and women would be enrolled in a vaccine trial, for example, HIV discordant couples. If a microbicide PrEP is tested, currently data exist to support only the use of a vaginal formulation. At the test-of-concept stage, PrEP trials initially had more stringent enrollment criteria, to exclude hepatic and renal dysfunction. Such restrictions would make enrollment of VAXPREP more difficult than an HIV vaccine alone trial. In a survey of men and women who were healthy by history and physical examination, from potential vaccine trial populations, 10–35% of volunteers would not be eligible.^{103,104} However, data from current PrEP test-of-concept trials may allow these criteria to be less stringent.

Adherence to PrEP is another challenge for investigators, unless it is administered in a long-acting formulation such as a depot injection or a vaginal ring. Participants, for example, may be tempted to share the study drug with their partner and other peers in their community, particularly if the availability of ART is limited. Counseling about and monitoring of drug sharing should therefore be tailored to this eventuality. Participants may discontinue taking their drug at some point due to study fatigue. The impact of PrEP may also be diminished or even reversed by behavioral disinhibition,

especially in scenarios with low coverage and low effectiveness.¹⁰⁵ Therefore, the individual impact of vaccine and PrEP on HIV infection may be difficult to distinguish during different periods in the study without a 2×2 factorial design. For example, if a trend toward diminishing efficacy is seen in the VAXPREP group over time, it will be difficult to determine if the drop is due to waning vaccine effect or waning PrEP adherence. Clues about decreasing the efficacy of VAXPREP may be found in a factorial design by examining efficacy between the VACCINE and PLACEBO groups and between the PREP and PLACEBO groups over time. Conversely, if PrEP adherence is high during the initial vaccination period, any vaccine-induced enhancement may be masked. In a factorial design, enhancement can be assessed by comparing the VACCINE and PLACEBO groups.

Finally, prior to undertaking VAXPREP trials, additional data would help guide trial design: estimates on adherence to oral or topical PrEP in populations targeted for AIDS vaccine trials, patterns of HIV seroconversion and HIV viral load observed in PrEP trials, and new guidelines for regulatory requirements, in particular for vaccine licensure. A vaccine showing efficacy only in combination with PrEP could be licensed only for use in combination with PrEP, which given the current economic limitation on ARV treatment in developing countries would be a significant concern. HIV-specific systemic and mucosal immune responses are being assessed in volunteers randomized to PrEP or placebo. These exploratory data on the timing and duration of the responses may influence the design of subsequent VAXPREP trials.

Lowering HIV viral load in genital secretions of HIV-infected individuals through the use of ART may improve the efficacy of preventive AIDS vaccines. Exposure to treated individuals would presumably result in a lower viral inoculum resulting in a lower transmission rate, as recently observed in injecting drug users in Canada.¹⁰⁶ The immune response elicited by a vaccine may be more efficient to counter a lower HIV viral "natural challenge." Even at low levels of population coverage and less than complete efficacy, together concurrent ART in HIV-infected individuals and a safe and efficacious preventive vaccine could markedly reduce the HIV-infected population over 20 years.¹⁰⁷

Interventions including microbicides, vaccines, and PrEP aimed at countering HIV infection should be given high priority for development.^{108,109} Evaluation of a possible interaction, such as synergy, between AIDS vaccines, PrEP, and ART for prevention could provide important information for development and rollout of AIDS vaccines and combination HIV prevention. If interactions occur, but are not understood, product development and public health decisions may not have the expected effects.

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Appendix: Statistical Considerations

For the two-arm study, we postulate that the combination of an AIDS vaccine and PrEP may reduce the risk of HIV infection or reduce the plasma viral load in those who become infected, relative to PrEP alone. In statistical terms, we treat HIV infection and viral load as coprimary endpoints and test a composite null hypothesis that the vaccine neither reduces the risk of HIV infection nor the viral load at setpoint. The composite null hypothesis is expressed as H_0 : $VEs = 0$ and $\Delta VL = 0$, where VEs is the percent reduction in the cumulative HIV incidence due to vaccination and ΔVL is the difference in average viral load measured on a $\log_{10}$ scale between vaccine recipients and placebo recipients who become infected. Following the approach of the Step trial,⁹⁵ we would test H_0 against the one-sided alternative H_1 : $VEs > 0$ or $\Delta VL > 0$.

For the endpoint of HIV infection, a test statistic Z_1 based on the difference in cumulative incidence and its corresponding one-sided p -value p_1 would be calculated. For the endpoint of viral load, a test statistic Z_2 based on the Wilcoxon rank sum test and its corresponding one-sided p -value p_2 would be computed. The two p -values are then combined using Fisher's method. If the resulting p -value is less than 0.05, preliminary evidence of efficacy is demonstrated and the vaccine candidate is considered for further efficacy testing.

Fisher's test gives equal weight to the infection and viral load endpoints. Therefore, VAXPREP could be declared efficacious over PrEP if either a significant reduction in the risk of HIV infection or a significant decrease in setpoint viral load is observed. If one endpoint is felt to be more important for making a decision to move VAXPREP forward into Phase III trials, a weighted version of Fisher's test could be employed in which the endpoint of greater importance is given greater weight. Another approach is to split the Type I error probability between the two endpoints. VAXPREP, for example, could be considered efficacious if the observed reduction in HIV incidence is significant at the 0.04 level or if the observed decrease in setpoint viral load is significant at the 0.01 level.

The approach used to control the Type I error probability, however, has implications for the size of the trial. If greater importance is placed on prevention of HIV infection, then the sample size will be larger than a trial that places more weight on the viral load endpoint.

In Table 1, VEs is defined as the percent reduction in the cumulative HIV incidence due to vaccination and as such equals the average vaccine efficacy over the follow-up period. If VEs (0–6 months) equals vaccine efficacy during the vaccination period and VEs (6–24 months) equals vaccine efficacy during the 18-month postvaccination follow-up period, then $VEs = 0.25 \cdot VEs$ (0–6 months) + $0.75 \cdot VEs$ (6–24 months). If VEs equals 50%, for example, and there is no benefit of vaccination during the vaccination period, then VEs (6–24 months) equals 67%. However, if the full effect of vaccination is achieved after one or two doses of vaccine, then VEs (6–24 months) will be closer to 50%.

For the 2×2 factorial trial, we test two related null hypotheses. The first is that the risk of infection and setpoint viral load is the same in the vaccine only group as that in the double placebo group. The second is that the risk of infection and setpoint viral load is the same in the VAXPREP group as that in the double placebo group. To maintain an overall type I error probability of 0.05, we suggest that the main effect of vaccination be assessed using a one-sided 0.025 level Fisher test as described for the two-arm trial. The overall benefit of vaccine with PrEP would similarly be assessed using a one-sided 0.025 level test. If the test of the main effect of vaccination is significant at the 0.025 level, then vaccine alone may be recommended for further testing. If the test of the combination is significant at the 0.025 level, then vaccine with PrEP may be recommended. If both tests are significant at the 0.025 level, then we may select VAXPREP over vaccine alone if the test of VAXPREP has the smaller p -value. Caution, however, is needed in the selection of VAXPREP over vaccine alone since the p -values are more likely to be influenced by the viral load endpoint than the HIV acquisition endpoint. Theoretically, VAXPREP could be more effective at reducing viral load.