

Improved Immunogenicity With High-Dose Seasonal Influenza Vaccine in HIV-Infected Persons

A Single-Center, Parallel, Randomized Trial

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Background: HIV-infected persons have less robust antibody responses to influenza vaccines.

Objective: To compare the immunogenicity of high-dose influenza vaccine with that of standard dosing in HIV-positive participants.

Design: Randomized, double-blind, controlled trial. (ClinicalTrials.gov: NCT01262846)

Setting: The MacGregor Clinic of the Hospital of the University of Pennsylvania, Philadelphia, from 27 October 2010 to 27 March 2011.

Participants: HIV-infected persons older than 18 years.

Intervention: Participants were randomly assigned to receive either a standard dose (15 mcg of antigen per strain) or a high dose (60 mcg/strain) of the influenza trivalent vaccine.

Measurements: The primary end point was the rate of seroprotection, defined as antibody titers of 1:40 or greater on the hemagglutination inhibition assay 21 to 28 days after vaccination. The primary safety end point was frequency and intensity of adverse events. Secondary end points were seroconversion rate (defined as a greater than 4-fold increase in antibody titers) and the geometric mean antibody titer.

Results: 195 participants enrolled, and 190 completed the study (93 in the standard-dose group and 97 in the high-dose group). The seroprotection rates after vaccination were higher in the high-dose group for the H1N1 (96% vs. 87%; treatment difference, 9 percentage points [95% CI, 1 to 17 percentage points]; $P = 0.029$), H3N2 (96% vs. 92%; treatment difference, 3 percentage points [CI, -3 to 10 percentage points]; $P = 0.32$), and influenza B (91% vs. 80%; treatment difference, 11 percentage points [CI, 1 to 21 percentage points]; $P = 0.030$) strains. Both vaccines were well-tolerated, with myalgia (19%), malaise (14%), and local pain (10%) the most frequent adverse events.

Limitations: The effectiveness of the vaccine in preventing clinical influenza was not evaluated. The number of participants with CD4 counts less than 0.200×10^9 cells/L was limited.

Conclusion: HIV-infected persons reach higher levels of influenza seroprotection if vaccinated with the high-dose trivalent vaccine than with the standard-dose.

Primary Funding Source: National Institute of Allergy and Infectious Diseases and Center for AIDS Research of the University of Pennsylvania.

Ann Intern Med. 2013;158:19-26.

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The emergence of a novel influenza A H1N1 subtype in the spring of 2009 and its subsequent spread to pandemic levels (as declared by the World Health Organization [1]) have highlighted the continuing threat of the influenza virus and recall the challenges of past pandemics. In each of these instances, human populations lacked substantial levels of preexisting immunity to the pandemic influenza virus, resulting in rapid spread and massively increased morbidity and mortality worldwide (1, 2).

Immunosuppressed persons are at particular risk for contracting the influenza virus and have been shown to have an increased risk for complications after influenza infections (3). Although data supporting a higher influenza incidence among HIV-infected persons are scant (4–6), several studies have shown greater hospitalization rates (7), extended illness (8, 9), and increased mortality (10) in this population. Recent epidemiologic studies have shown similar patterns with the pandemic H1N1 strain among HIV-infected persons (11, 12). This has been a particularly worrisome trend in South Africa, where 53% of the deaths from H1N1 over 7 months in 2009 had underlying HIV infection (13). Furthermore, data from Mexico suggest that patients with CD4 counts less than 0.200×10^9 cells/L or an opportunistic infection are at increased risk

for complications requiring inpatient care (relative risk, 24.5) and mechanical ventilation (relative risk, 19.7) (14).

In healthy adults, the inactivated seasonal influenza vaccine provides an estimated protective efficacy of 70% to 90% (15). This correlates with levels of antibody to viral receptor hemagglutinin, as measured by the serum hemagglutination inhibition (HI) assay, with titers of 1:40 or greater corresponding to relative protection from the influenza virus (16, 17). Several studies have shown that antibody responses after seasonal influenza immunization in HIV-infected persons are lower than in the general population, although this finding has not been consistent across all studies (18–24). Data from our recent study suggest similar difficulties in attaining protection with the pandemic H1N1 influenza vaccine, with only 61% (95% CI, 51% to 71%) of HIV-infected persons developing protective titers (25). The main predictors of vaccine response in this population have been CD4 cell count and the presence of HIV viremia (26–28). Despite poor seroconversion rates, the general recommendation is yearly influenza vaccination for all HIV-infected patients with the dose level of 15 mcg of antigen per strain found in the standard influenza vaccines (29, 30).

Context

Compared with the general population, HIV-infected patients may have greater morbidity and mortality from influenza and a decreased response to seasonal influenza vaccine.

Contribution

In a randomized, controlled trial, HIV-infected patients who received a quadruple dose of seasonal influenza vaccine had a higher antibody response and greater seroconversion rate than did those who received a standard dose. Adverse event rates were similar in the 2 intervention groups.

Caution

The trial was not powered to determine clinical efficacy or to detect differences in the occurrence of less common adverse events.

Implication

Administering a quadruple dose of seasonal influenza is a possible approach to seasonal influenza vaccination of HIV-infected patients.

—The Editors

It is necessary to evaluate strategies that increase the efficacy of influenza vaccination in HIV-infected persons. Higher doses of antigen have been associated with higher antibody titers in other poorly responsive populations, such as elderly adults (31–33). We hypothesized that increasing the antigen dose with the recently approved Fluzone High-Dose vaccine (Sanofi Pasteur, Bridgewater, New Jersey) would lead to improved immunogenicity in HIV-infected persons.

METHODS**Design Overview**

We conducted a single-site, double-blind, randomized, controlled trial comparing the immunogenicity of a high-dose (HD) influenza vaccine with the standard dose (SD) in HIV-infected persons. The vaccines were compared in parallel, with a 1:1 allocation ratio of participants to each study group.

Setting and Participants

HIV-infected persons older than 18 years who had an indication for receiving the influenza vaccine were eligible for recruitment. Other inclusion criteria were that participants had to be receiving stable antiretroviral therapy (ART) (as outlined in the U.S. Department of Health and Human Services treatment guidelines for HIV-1-infected persons [34]) or not receiving ART and not intending to start it in the next 30 days. We excluded persons with a known allergy to eggs or other components of the vaccine and a history of severe reactions to previous immunization

with seasonal influenza, as well as previous recipients of the 2010–2011 seasonal influenza vaccine. We also excluded recipients of other licensed live vaccines within 4 weeks or inactivated vaccines within 1 week of study entry. Patients who were receiving experimental treatments, systemic chemotherapy for the previous 36 months, or steroids and other immunomodulators or who had a history of the Guillain-Barré syndrome were excluded.

The study was done at the MacGregor Clinic of the Hospital of the University of Pennsylvania in Philadelphia. Sequential clinic patients who agreed to participate were enrolled. We recruited the first patient on 27 October 2010 and completed the last follow-up on 27 March 2011. All patients provided informed consent. The study was approved by the University of Pennsylvania Institutional Review Board and registered at ClinicalTrials.gov.

Randomization and Interventions

We performed simple unrestricted randomization by using a computerized random-number generator. Eligible patients were randomly assigned in equal numbers to the 2 intervention groups, with no stratification. The study pharmacist who prepared the vaccines for administration kept the allocation sequence secured and was the only team member aware of each participant's allocation. The rest of the study team and the patients were blinded to the vaccine assignment.

Vaccine

For the HD study group, we used a licensed HD formulation of a trivalent, inactivated, unadjuvanted influenza vaccine for intramuscular use, prepared from influenza viruses propagated in embryonated chicken eggs (Fluzone High-Dose, lot 37756AA). Each 0.5-mL dose contained 60 mcg of hemagglutinin from the following strains: A/California/07/2009 X-179A (H1N1), A/Victoria/210/2009 X-187 (H3N2 [an A/Perth/16/2009-like virus]), and B/Brisbane/60/2008 (influenza B). Patients randomly assigned to the SD group received a licensed trivalent influenza vaccine (Fluzone, lot U32347DA), with each 0.5-mL dose containing 15 mcg of antigen from each of the previously listed strains.

Each participant had baseline studies measuring CD4 cell count and HIV viral load before enrollment. The HD or SD vaccine was then administered intramuscularly in one of the deltoid muscles. Patients and staff administering the vaccine were blinded to group assignment and type of vaccine dispensed. Vaccine needles and syringes were identical and had opaque stickers affixed over the label to conceal the formulation.

Outcomes and Follow-up

The primary outcome measure of immunogenicity for the study was the proportion of participants with seroprotective antibody levels at 21 to 28 days after vaccination (defined as antibody titers \geq 1:40 on the HI assay). The primary safety end point was the frequency and intensity of adverse events up to 28 days after vaccination (the day of

the final visit). Secondary end points included seroconversion rate (defined as either an increase in antibody titers from $<1:10$ to $\geq 1:40$ at 21 to 28 days after vaccination or a >4 -fold increase in antibody titers after vaccination from a prevaccination titer $>1:10$) and the geometric mean titers (GMTs) of the HI assay before and after receiving the vaccine. We used the GMTs of duplicate results for each specified time point for all calculations.

Immunologic Assessments

We measured antibodies to the influenza virus at baseline and at days 21 to 28 after immunization by using the HI assay against the 3 components of the vaccine: the H1N1, H3N2, and influenza B strains. The analysis was done blinded and in duplicate for each time point at Sanofi Pasteur's Global Clinical Immunology Laboratory in Swiftwater, Pennsylvania.

The HI assay was done according to established protocols. Briefly, sera were treated with receptor-destroying enzymes by diluting 1 part serum with 4 parts enzyme and were incubated overnight at 37 °C in a water bath. The enzyme was then inactivated by 60-minute incubation at 56 °C, followed by adsorption with erythrocytes and addition of phosphate buffer saline for a final dilution of 1/10. Assays were done in V-bottom 96-well plates by using 4 hemagglutinin units of virus per 25 μL and 0.5% turkey erythrocytes.

Safety Assessments

We collected data on local and systemic adverse events until the postvaccination visit (21 to 28 days) by using a modified HIV Vaccine Trials Network questionnaire, which has been validated by the network. We contacted participants by telephone on the fourth and tenth days after vaccination and asked about any adverse effects they may have had from the vaccine. The questionnaire included a checklist of common adverse effects and an open-ended section for participants to relay any symptoms not included on the checklist. These same questions were asked in person on the follow-up visit. We used the standard AIDS Clinical Trials Group grading scale to evaluate adverse events.

Statistical Analysis

We used the statistical software SPSS, version 20.0 (SPSS, Chicago, Illinois), for data analyses. A *P* value less than 0.05 was considered to be statistically significant.

We based sample size calculations on a previous study by our group and similar studies where the seroprotection rate in HIV-infected patients after vaccination with the H1N1 2009 vaccine was 55% (25, 35). We selected the sample size for this study to demonstrate the primary outcome of vaccine superiority in the HD group. A total sample size of 232 was planned, with an anticipation that 20% of participants would not return for the second visit at 21 to 28 days. A sample size of 192 evaluable participants (96 per group) had a 90% power to detect a 20% improvement in the serologic response with the new HD vaccine

(G*Power, Version 3.2.1, Kiel, Germany). For the safety end points, finding no grade 3 or grade 4 adverse events in a total evaluable sample of 96 patients in each group provided 95% confidence that the rate in the population from which the sample was drawn was no greater than 3%. When 192 participants completed the last visit of the study, enrollment was discontinued.

For the baseline characteristics, we present continuous variables as medians and interquartile ranges and categorical variables as frequencies and percentages. For the primary immunogenicity and safety outcomes and secondary immunogenicity outcomes, estimates of proportions and exact 95% binomial CIs and 95% CIs for the differences between the groups (proportions and GMT ratio) were calculated. We used the chi-square test to evaluate the significance of the differences between groups. For GMT and mean fold increase in GMT, we calculated the mean and 95% CI for the \log_{10} -transformed titers and then transformed back to the original units by exponentiation. We used the *t* test to compare the GMTs between groups.

To explore what other variables may be associated with seroconversion, we ran logistic regression models separately for each strain. We included variables that have been associated previously with vaccine responses in HIV-infected persons: baseline HI antibody titer for the corresponding strain, randomization to the HD group, HIV viral load less than the limit of detection, current and nadir CD4 cell count, sex, and receipt of ART. All of the variables were entered into the models.

Role of the Funding Source

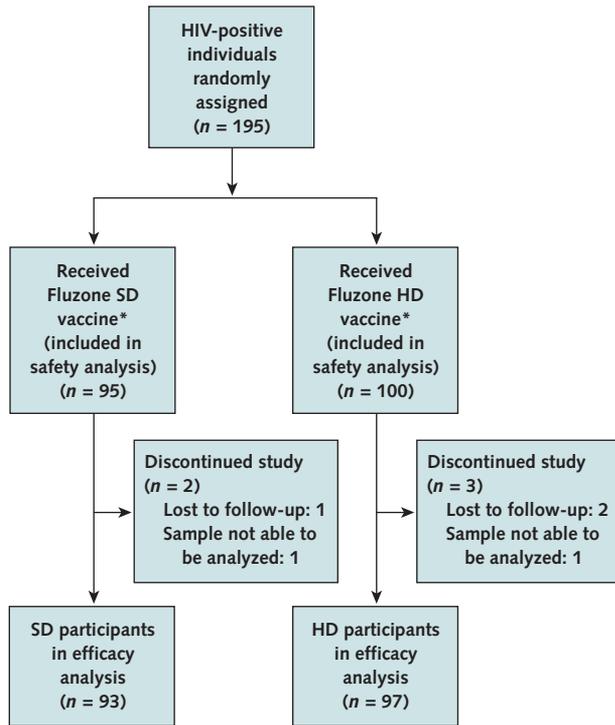
The National Institute of Allergy and Infectious Diseases and the Center for AIDS Research of the University of Pennsylvania supported the study. The funding source did not have any role in the design of the study, its administration, or the analysis of the results and was not involved in manuscript preparation or submission.

RESULTS

From 27 October 2010 to 27 March 2011, we enrolled 195 participants. Of these, 192 vaccinated participants completed the second visit—182 within the 21-to-28-day window and 10 outside of the window (range, 29 to 45 days). There were 2 participants whose specimens could not be processed. All 195 participants are included in the safety analysis, and 190 participants are included in the immunogenicity analysis (Figure).

The participants' baseline demographic and clinical characteristics are summarized in Table 1. Data from both groups show that participants were mostly men (77% in the SD group and 64% in the HD group) and African American (61% in the SD group and 78% in the HD group). Most patients in both groups were receiving ART (88% in the SD group and 90% in the HD group), and most of the participants receiving ART had an HIV RNA viral load less than 400 copies/mL (89% in the SD group

Figure. Study flow diagram.



HD = high dose; SD = standard dose.

* Fluzone is manufactured by Sanofi Pasteur (Bridgewater, New Jersey).

and 88% in the HD group). Eighty-one percent of patients in the SD group and 74% of patients in the HD group who were receiving ART had HIV viral loads less than the limit of quantification of an ultrasensitive assay. The me-

dian nadir CD4 count among patients in the SD group was 0.166×10^9 cells/L and the median current CD4 count was 0.453×10^9 cells/L. For patients in the HD group, the median nadir CD4 count was 0.174×10^9 cells/L and the median current CD4 count was 0.438×10^9 cells/L. Approximately 10% of the patients had current CD4 counts less than 0.200×10^9 cells/L. Roughly three quarters of the participants had received the regular trivalent vaccine during the 2009–2010 season, and half had been vaccinated against the epidemic H1N1 strain the prior year.

At the baseline evaluation, 52% of SD recipients and 49% of HD recipients had seroprotective antibody titers ($\geq 1:40$) for the H1N1 strain. Baseline seroprotective rates were 52% in the SD group and 44% in the HD group for the H3N2 strain and 52% in the SD group and 48% in the HD group for the influenza B strain.

Prevaccination GMTs were similar between the 2 groups for each of the 3 antigens. Immunogenicity analysis at the second evaluation showed that for the H1N1 strain, the postvaccination GMTs were 344 (CI, 229 to 518) in the SD group and 686 (CI, 509 to 926) in the HD group (Table 2). For the H3N2 strain, GMTs were 324 (CI, 227 to 464) in the SD group and 739 (CI, 529 to 1032) in the HD group. The GMTs for the influenza B strain were 64 in the SD group (CI, 46 to 91) and 140 in the HD group (CI, 110 to 178). For all of these comparisons, the HD vaccine was statistically more immunogenic than the SD vaccine.

The percentage of persons with seroprotective antibody titers for the 3 virus strains was greater among those who received the HD vaccine; significant ($P < 0.05$) increases were seen for the H1N1 and influenza B strains

Table 1. Baseline Characteristics

Characteristic	SD Recipients (n = 95)	HD Recipients (n = 100)
Median age (IQR), y	46 (37 to 53)	44 (35 to 50)
Male, n (%)	73 (77)	64 (64)
Race/ethnicity, n (%)		
Asian/Pacific	1 (1)	0 (0)
Black	58 (61)	78 (78)
Hispanic	7 (7)	3 (3)
White	36 (38)	22 (22)
Receiving ART, %	88	90
HIV RNA load <400 copies/mL, %	89	88
HIV RNA load less than limit of detection, %	81	74
Median nadir CD4 count (IQR), $\times 10^9$ cells/L	0.166 (0.037 to 0.278)	0.174 (0.045 to 0.343)
Median current CD4 count (IQR), $\times 10^9$ cells/L	0.453 (0.301 to 0.660)	0.438 (0.275 to 0.625)
Current CD4 count < 0.200×10^9 cells/L, n (%)	8 (8)	14 (14)
Vaccinated in 2009–2010, n (%)		
Trivalent	65 (68)	76 (76)
H1N1	37 (39)	47 (47)
Baseline HI antibody titer $\geq 1:40$, n (%)*		
H1N1	49 (52)	49 (49)
H3N2	49 (52)	44 (44)
Influenza B	49 (52)	48 (48)

ART = antiretroviral therapy; HD = high dose; HI = hemagglutination inhibition; IQR = interquartile range; SD = standard dose.

* N = 190. Only participants included in the efficacy analysis had baseline titers available.

Table 2. Pre vaccination and Postvaccination GMTs

Virus Strain	Day	HI GMTs (95% CI)		GMT Ratio (HD–SD) (95% CI)	P Value
		SD Recipients (n = 93)	HD Recipients (n = 97)		
H1N1 (A/California/07/2009 X-179A)	0	22 (14 to 37)	25 (15 to 40)	1.1 (0.4 to 1.8)	–
	21	344 (229 to 518)	686 (509 to 926)	2.0 (1.2 to 3.3)	0.008
H3N2 (A/Victoria/210/2009 X-187)	0	25 (16 to 42)	26 (16 to 42)	1.0 (0.5 to 1.8)	–
	21	324 (227 to 464)	739 (529 to 1032)	2.3 (1.4 to 3.7)	0.001
Influenza B (B/Brisbane/60/2008)	0	17 (11 to 25)	20 (14 to 28)	1.2 (0.5 to 1.4)	–
	21	64 (46 to 91)	140 (110 to 178)	2.2 (1.4 to 3.3)	<0.001

GMT = geometric mean titer; HD = high dose; HI = hemagglutination inhibition; SD = standard dose.

(Table 3). At weeks 3 to 4, the HD vaccine was more immunogenic than the SD vaccine for the H1N1 strain (treatment difference, 9 percentage points [CI, 1 to 17 percentage points]; $P = 0.029$). The difference favoring the HD vaccine was 3 percentage points (CI, -3 to 10 percentage points; $P = 0.32$) for the H3N2 strain and 11 percentage points (CI, 1 to 21 percentage points; $P = 0.030$) for the influenza B strain. Seroconversion rates were also greater in the HD group than in the SD group for the H1N1 (75% vs. 59%), H3N2 (78% vs. 74%), and influenza B (56% vs. 34%) strains.

In an exploratory analysis, participants with current CD4 counts less than 0.200×10^9 cells/L seemed to be less responsive to both the HD and SD influenza vaccinations. Among the 14 participants in the HD group with CD4 counts less than 0.200×10^9 cells/L, fewer developed seroprotective levels than did those with CD4 counts greater than 0.200×10^9 cells/L (93% vs. 96% for H1N1, 79% vs. 99% for H3N2, and 64% vs. 95% for influenza B). The seroprotective response rates among the participants receiving the HD vaccine tended to be higher than among those receiving the SD vaccine (Appendix Table 1, available at www.annals.org).

In logistic regression models built separately for each strain, baseline HI antibody titer for the corresponding strain and randomization to the HD group were always associated with seroconversion. HIV viral load less than the limit of detection, current and nadir CD4 cell count, sex, and receipt of ART were not independently associated with

seroconversion in these models (Appendix Table 2, available at www.annals.org).

Both vaccines were well-tolerated, with grade 1 to grade 2 local reactions at the injection site elicited in less than 20% of the participants and no differences between the 2 study groups. Mild systemic adverse events (headache, malaise, and myalgia) were noted in 10% to 20% of the participants. There was no significant difference in the local or systemic reactions between the 2 groups and no serious adverse events related to vaccine administration. Three participants were hospitalized during the study (for pancreatitis, candidiasis, and right lower extremity cellulitis [not at the injection site]); these hospitalizations were considered unrelated to the vaccine. Table 4 summarizes adverse events reported. The most frequent local adverse events were pain and tenderness at the injection site. The most frequent systemic adverse effect was myalgia, followed by malaise and headache.

DISCUSSION

The results from this randomized, controlled study suggest that 1 way to increase the protective antibody titers for influenza in HIV-infected persons is to administer a higher dose (60 mcg/antigen—4 times the standard dose of 15 mcg/antigen) of the trivalent seasonal influenza vaccine. For every antigen studied, the HD formulation resulted in an increased average antibody titer and higher seroconversion and seroprotective rates compared with the

Table 3. Seroconversion and Seroprotection Rates After Vaccination*

Variable	Type of Vaccine	Proportion (95% CI)		Difference (95% CI), percentage points	P Value
		SD Recipients (n = 93)	HD Recipients (n = 97)		
Seroconversion	H1N1	59 (49 to 69)	75 (67 to 84)	16 (3 to 29)	0.018
	H3N2	74 (65 to 83)	78 (70 to 87)	4 (-8 to 16)	0.50
	Influenza B	34 (25 to 44)	56 (46 to 66)	21 (7 to 35)	0.003
Seroprotection	H1N1	87 (80 to 94)	96 (92 to 100)	9 (1 to 17)	0.029
	H3N2	92 (87 to 98)	96 (92 to 100)	3 (-3 to 10)	0.32
	Influenza B	80 (71 to 88)	91 (85 to 97)	11 (1 to 21)	0.030

HD = high dose; SD = standard dose.

* Seroconversion is defined as a 4-fold increase in antibody titer from baseline or an increase in titer from $\leq 1:10$ to $1:40$. Seroprotection is defined as an antibody titer $\geq 1:40$.

Table 4. Adverse Events During the Trial*

Adverse Event	SD Recipients (n = 95)	HD Recipients (n = 100)
Serious adverse event, n (%)†	3 (3.2)	0 (0.0)
Death	0 (0.0)	0 (0.0)
Hospitalization	3 (3.2)	0 (0.0)
Patients with ≥1 adverse event, n (%)	76 (80.0)	90 (90.0)
Local adverse events, n (%)‡	16 (16.8)	30 (30.0)
Pain	4 (4.2)	15 (15.0)
Redness	1 (1.1)	3 (3.0)
Induration	1 (1.1)	2 (2.0)
Tenderness	10 (10.5)	10 (10.0)
Systemic adverse events, n (%)‡	57 (60.2)	60 (60.0)
Arthralgia	3 (3.2)	5 (5.0)
Chills	3 (3.2)	5 (5.0)
Diarrhea	1 (1.1)	0 (0.0)
Nausea	8 (8.4)	7 (7.0)
Headache	9 (9.5)	8 (8.0)
Lymphadenopathy	1 (1.1)	0 (0.0)
Malaise	14 (14.7)	13 (13.0)
Myalgia	17 (17.9)	19 (19.0)
Vomiting	1 (1.1)	3 (3.0)

HD = high dose; SD = standard dose.

* Events were recorded in telephone interviews on the fourth and tenth days after vaccination and on a questionnaire completed during the follow-up visits on days 21 to 28.

† Hospitalizations were due to esophageal candidiasis, right lower extremity cellulitis, and pancreatitis. All events were in the SD group and were determined to be unrelated to the vaccine intervention.

‡ The number of patients with a specific adverse event at least once during the study is shown. A patient could have more than 1 adverse event.

SD influenza vaccine. The HD vaccine was also well-tolerated, with adverse event rates similar to those of the SD vaccine. Our exploratory analysis suggests that the HD vaccine may also be more immunogenic than standard dosing in patients with CD4 counts less than 0.200×10^9 cells/L.

Although rates of influenza vaccination have been increasing in high-risk populations, such as HIV-infected persons, data from previous studies suggest that, independent of virologic control and CD4 recovery, as many as 40% of HIV-positive persons do not reach seroprotective antibody levels after vaccination (36, 37). This suggests that the current influenza vaccination strategy for this group may be inadequate, given the increased morbidity and mortality associated with influenza infection in this population (38).

Alternative dosing strategies have been tried in other studies. Two different studies have evaluated the use of higher doses of influenza vaccine to improve vaccine efficacy in HIV-infected persons. One such study was done by Cooper and colleagues (39) at 12 Canadian HIV Trials Network sites. That trial assessed 3 dosing strategies for influenza vaccine in HIV-infected adults: 2 standard doses (15 mcg) over 28 days, 2 double doses (30 mcg) over 28 days, and a single standard dose. Even with the increased antigen dose and booster dosing, unadjuvanted influenza

vaccine immunogenicity was poor, with rates of seroconversion in the 50% range. The second study, done by El Sahly and colleagues (40), compared the use of 1 or 2 doses of 15 mcg or 30 mcg hemagglutinin of the 2009 H1N1 vaccine given 21 days apart to participants stratified by baseline CD4 cell count. The higher dose was more immunogenic, particularly among persons with CD4 counts less than 0.200×10^9 cells/L; however, the percentage of participants with seroprotective antibody titers 3 weeks after the second dose was still less than the desirable levels (74% and 69%, respectively, of the participants with a high and low CD4 cell count who were receiving the HD vaccine had titers $\geq 1:40$). In that study, the addition of a second 30-mcg dose did not clearly improve the immunogenicity.

Another strategy has been the use of adjuvants, such as ASO3 and MF59. Two doses of the ASO3 adjuvanted 2009 H1N1 vaccine and a single dose of the MF59 adjuvanted vaccine resulted in immune responses in HIV-infected adults similar to those in healthy adults (41, 42). A second dose in the MF59 study had only modest additional effects. Currently, however, the use of these adjuvants is not approved in many countries, including the United States.

The main limitation of our study (and many influenza vaccine trials in immunosuppressed populations) is that we measured serologic responses rather than the incidence of clinical influenza, which would require a much larger sample size. Other immunogenicity measurements, such as virus microneutralization, interferon- γ enzyme-linked immunospot, or proliferation assays, would provide good corollary information but were not done because of budget limitations.

Most of the patients in our practice are receiving ART and have an undetectable HIV RNA viral load and a relatively high CD4 cell count. Our study therefore had limited power to detect different effects of the 2 vaccines among participants with ongoing HIV viremia or low CD4 cell counts—arguably the populations most at risk for morbidity and mortality from influenza. However, the higher dose of the influenza vaccine was more immunogenic in these 2 populations, and there is no biologically plausible reason to believe that the standard dose would prove to be more efficacious than the higher dose in these poorly reactive groups in a larger study.

Half of the participants in this study had evidence of protective titers at baseline, which was probably the result of an increased rate of vaccination associated with the 2009–2010 pandemic and the fact that HIV-infected persons are more frequently engaged in care than are other underserved populations. This high baseline seropositivity rate may have contributed to the very high seroprotection rate at the end of our study.

The implications of this research are important for future vaccination efforts in the HIV-positive population. This study suggests that a substantial number of HIV-

infected patients may not be obtaining sufficient protection with the standard influenza vaccine. A strategy with a single HD immunization is much easier to implement than a multiple-dose schedule (38). Although a higher dose is 1 route to the protection of this vulnerable population, other strategies may also be explored in the future, such as alternative vaccines, the use of adjuvants, or new schedule strategies.

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Disclaimer: Sanofi Pasteur was not involved in the study design or the analysis.

Grant Support: In part by grant U01-AI069467 from the National Institute of Allergy and Infectious Diseases and grant P30-AI045008 from the Center for AIDS Research to the University of Pennsylvania.

Potential Conflicts of Interest: Disclosures can be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M12-1043.

Reproducible Research Statement: *Study protocol, statistical code, and data set:* Available from Dr. Tebas (e-mail, pablo.tebas@uphs.upenn.edu).

Requests for Single Reprints: Pablo Tebas, MD, University of Pennsylvania, AIDS Clinical Research Unit, 502 Johnson Pavillion, 3610 Hamilton Walk, Philadelphia, PA 19104.

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Appendix Table 1. Seroconversion and Seroprotection Rates in Participants With CD4 Counts Less Than 0.200×10^9 cells/L

Variable	Type of Vaccine	Proportion (95% CI)		Difference (95% CI), percentage points	P Value
		SD Recipients (n = 8)	HD Recipients (n = 14)		
Seroconversion	H1N1	50 (13 to 87)	79 (56 to 100)	29 (–12 to 69)	0.22
	H3N2	50 (13 to 87)	71 (47 to 96)	21 (–21 to 64)	0.36
	Influenza B	25 (0 to 57)	36 (10 to 62)	11 (–31 to 53)	0.61
Seroprotection	H1N1	75 (43 to 100)	93 (79 to 100)	18 (–12 to 48)	0.34
	H3N2	50 (13 to 87)	79 (56 to 100)	29 (–12 to 69)	0.22
	Influenza B	36 (2 to 73)	64 (38 to 90)	27 (–17 to 71)	0.26

HD = high dose; SD = standard dose.

Appendix Table 2. Logistic Regression Model Data

Variable	H1N1		H3N2		Influenza B	
	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
HD vs. SD vaccine	2.906 (1.427–5.918)	0.003	2.828 (1.455–5.499)	0.002	2.828 (1.455–5.499)	0.002
Undetectable HIV viral load	0.986 (0.619–1.570)	0.95	0.688 (0.419–1.129)	0.139	0.688 (0.419–1.129)	0.139
Sex	2.124 (0.965–4.674)	0.061	0.762 (0.362–1.603)	0.47	0.762 (0.362–1.603)	0.47
Race	0.823 (0.379–1.785)	0.62	1.354 (0.653–2.806)	0.42	1.354 (0.653–2.806)	0.42
Current CD4 cell count	1.002 (1.000–1.003)	0.074	0.999 (0.998–1.001)	0.43	0.999 (0.998–1.001)	0.43
Nadir CD4 cell count	0.998 (0.996–1.001)	0.20	1.000 (0.998–1.002)	0.98	1.000 (0.998–1.002)	0.98
Receiving ART	2.087 (0.613–7.107)	0.24	0.963 (0.288–3.225)	0.95	0.963 (0.288–3.225)	0.95
Baseline seronegativity*	0.474 (0.334–0.673)	<0.001	0.438 (0.280–0.686)	<0.001	0.438 (0.280–0.686)	<0.001

ART = antiretroviral therapy; HD = high dose; SD = standard dose.

* For antigen of interest.