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A Phase III, randomized study to evaluate the immunogenicity and safety of an MF59®-adjuvanted A/H1N1 pandemic influenza vaccine in HIV-positive adults ☆

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ABSTRACT

Background and aims: Antibody responses to vaccines are suboptimal in immunosuppressed HIV-positive individuals. This study aimed to evaluate the potential benefits of MF59® adjuvant or a second A/H1N1 influenza vaccine dose in HIV-positive adults.

Method: HIV-positive adults ($n = 61$) and HIV-negative controls ($n = 93$) aged 18–60 years received two doses of A/H1N1, either as MF59-adjuvanted A/H1N1 pandemic vaccine, or as part of a unadjuvanted seasonal influenza vaccine containing the pandemic strain. Immunogenicity was assessed against the vaccine strain, A/California/7/2009, by haemagglutination inhibition (HI) assay three weeks after the administration of each vaccine dose. Local and systemic reactions were recorded for three days after each vaccination. Unsolicited adverse events were recorded throughout the six-week study period.

Results: Both adjuvanted and unadjuvanted vaccines met the European licensure criteria in HIV-positive and HIV-negative study groups after a single dose. Lower antibody titres were observed with both adjuvanted and unadjuvanted vaccine in HIV-positive compared to HIV-negative subjects. A second dose of either vaccine did not compensate for the lower response of HIV-infected subjects. In HIV-positive subjects, $CD4^+$ T cell counts and levels of CD38 expression on $CD8^+$ T cells remained stable throughout the study period. Both vaccine formulations were generally well tolerated, with no increased reactogenicity observed in response to the adjuvanted vaccine.

Conclusion: Antibody responses in HIV-positive subjects were acceptable but lower than those in healthy control subjects, whether subjects were immunized with one or two doses of adjuvanted or unadjuvanted vaccine. Vaccination did not affect rates of HIV replication, $CD4^+$ T cells counts, or levels of CD38 expression among patients under successful antiretroviral treatment.

1. Introduction

When immunosuppressed, human immunodeficiency virus (HIV)-positive individuals are at increased risk for influenza-related morbidity, and are therefore, among the high-risk groups recommended to receive seasonal influenza vaccine on an annual basis; for similar reasons, during the 2009 A/H1N1 pandemic, they were prioritized to receive the pandemic vaccine [1,2]. Although vaccination against seasonal influenza has reduced incidences of influenza illness, complications, and the overall risk of hospitalization in HIV-positive individuals [3,4], antibody responses to vaccination in this population are in general less robust than those seen in HIV-negative, healthy adults [2,5–8]. HIV-positive individuals also produced suboptimal antibody responses to pandemic A/H1N1 influenza vaccination, which were attributed to various factors including age, receipt of antiviral therapy, low $CD4^+$ T cell counts, high viral loads, high baseline antibody titres, and previous vaccination against seasonal influenza [9–15]. Of concern, is the effect of influenza vaccination on HIV viral replication [16] and less clear is the effect on T cell activation among individuals under successful HAART.

Abbreviations: CD, cluster of differentiation; HI, haemagglutination inhibition; HIV, human immunodeficiency virus; CHMP, Committee for Medicinal Products for Human Use; AE, adverse event; SAE, serious adverse event; GMR, geometric mean ratio; GMT, geometric mean titre; CI, confidence interval.

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Oil-in-water emulsion adjuvants increase the immunogenicity of influenza vaccines, and in HIV-infected persons, MF59®-adjuvant has provided variably higher antibody titres to seasonal antigens compared with unadjuvanted trivalent influenza vaccine (TIV). Since emulsion adjuvants were shown to increase the antibody responses to A/H5N1 vaccines, many countries deployed adjuvanted formulations of A/H1N1 pandemic vaccine. It was not known at the initiation of clinical trials whether one or two vaccine doses would be required for HIV-positive subjects. While other studies have compared responses of HIV-positive and HIV-negative control subjects to various pandemic vaccines, no other studies to our knowledge have simultaneously compared responses of both groups to both unadjuvanted and adjuvanted vaccines containing A/H1N1 pandemic antigen.

This Phase III, randomized trial evaluated the immunogenicity and acute safety of monovalent MF59-adjuvanted A/H1N1 pandemic influenza vaccine, and that of an unadjuvanted, seasonal TIV containing the A/H1N1 pandemic antigen in HIV-positive adults compared to HIV-negative control subjects. This study also aimed to identify whether a one- or two-dose vaccination schedule was most suitable for HIV-infected individuals. Possible vaccine induced changes in $CD4^+$ T cell counts, viral loads, and the expression of CD38 on $CD8^+$ T cells were assessed. Immunogenicity was analysed according to the European licensure criteria for pandemic influenza vaccines.

2. Materials and methods

2.1 Study design and objectives

This Phase III, randomized, controlled, open-label trial was conducted in HIV-positive and healthy HIV-negative adults, at two study sites in Brazil between April 2010 and June 2011. This was the first year the H1N1 vaccination was available in Brazil. The protocol was approved by the Brazilian National Research Ethics Committee and National Sanitary Surveillance Agency of Brazil. The trial was carried out in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. Before enrolment, written informed consent was obtained from each participant. The primary objective of this study was to compare the immunogenicity of two doses of MF59®-adjuvanted pandemic influenza vaccine compared with two doses of unadjuvanted, split, seasonal influenza vaccine (2010 southern hemisphere formulation containing A/California/07/09 [H1N1] antigen) in HIV positive individuals versus HIV uninfected individuals. HIV uninfected individuals were selected to match the same demographic characteristics as HIV infected individuals. The safety profiles of both vaccine formulations were assessed. HIV-positive and HIV-negative subjects were randomized in equal numbers to receive two doses of either MF59-adjuvanted A/H1N1 vaccine (aH1N1pnd) or unadjuvanted TIV, given three weeks apart. Blood samples were collected for immunogenicity analysis at baseline (Day 1), and three weeks after administration of the first (Day 22) and second (Day 43) vaccine doses. HIV-positive subjects had two additional blood draws on Days 8 and 29 for $CD4^+$ and $CD8^+$ T cell analyses.

2.2 Subjects

A total of 61 HIV-positive and 93 HIV-negative adult (18–60 years) subjects were enrolled. The inclusion criteria for HIV positive subjects were: confirmed HIV-1 infection; $CD4^+$ T cell count >200 cells/mm^3; HIV-1 viral load <200 copies/mL; no immunomodulatory therapy within three months prior to enrolment; and no changes in antiviral therapy (including HAART) four weeks prior to enrolment or planned within three weeks of second vaccination. The exclusion criteria were: laboratory confirmed A/California/7/2009 (H1N1) influenza infection; receipt of any vaccine or investigational agent within three and twelve months of enrolment, respectively; acute febrile illness; allergy to egg or egg protein; pregnancy; viral load >500 copies/mL within six months prior to study enrolment for HIV positive subjects; a history of cancer (except for skin cancer); and a history of cognitive or neurological disorders.

2.3 Vaccines

Each 0.5 mL dose of the investigational, MF59-adjuvanted, egg-derived, monovalent, pandemic influenza vaccine, Focetria® (Novartis Vaccines and Diagnostics, Siena, Italy; Lot.091001), contained 7.5 μg of A/California/7/2009 (H1N1) haemagglutinin and a standard quantity of MF59 adjuvant (9.75 mg squalene, 1.18 mg polysorbate 80, 1.18 mg sorbitan trioleate, 0.66 mg sodium citrate dehydrate, and 0.04 mg citric acid monohydrate). One 0.5 mL dose of the unadjuvanted, seasonal, trivalent, influenza vaccine, Begrivac® (Novartis Vaccines and Diagnostics, Marburg, Germany; Lot 226011C), contained 15 μg of antigen from each of the WHO recommended viral strains for the 2010–11 influenza season (southern hemisphere): A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008. Vaccines were supplied in prefilled, monodose-syringes and were administered intramuscularly in the deltoid muscle of the non-dominant arm.

2.4 Immunogenicity analysis

Blood samples of 10–15 mL and 10–25 mL were collected from HIV-negative and HIV-positive subjects, respectively. Sera were stored at −18 °C and shipped to the Novartis Vaccines Clinical Serology Laboratory in Marburg, Germany, where antibody responses were assessed by haemagglutination inhibition (HI) assay against the A/H1N1 pandemic vaccine antigen strain only. The HI assay was based on the method of Stephenson and colleagues [15]. HI titre was expressed as the reciprocal of the highest dilution at which haemagglutination was totally inhibited. Seroconversion was defined as a negative pre-vaccination antibody titre of <10 to a positive post-vaccination titre of $\geqslant$40, or as a $\geqslant$4-fold increase in subjects seropositive (titre $\geqslant$10) at baseline. HI titres below the detection limit of 10 were arbitrarily assigned to half that limit for the purpose of analysis.

2.5 Safety analysis

Subjects were observed for a minimum of 30 min after each vaccination to monitor for any possible immediate adverse reactions. Solicited local and systemic reactions were recorded in diary cards for three days after each vaccination. Local reactions included pain, and swelling or redness at the site of injection. Systemic reactions included fever (>37.5 °C), headache, muscle pain, irritability, sleepiness, loss of appetite, nausea, vomiting, urticaria, and rhinorrhea. All unsolicited adverse events (AEs), including serious adverse events (SAEs) and AEs of special interest (neuritis, seizures, severe allergic reactions, angioedema, non-infectious encephalitis, vasculitis, Guillain-Barré syndrome, demyelination, and Bell's palsy), were recorded throughout the entire study period (Day 1–43).

2.6 HIV-1 viral loads, $CD4^+$ T cell counts, and CD38 expression on $CD8^+$ T cells

HIV-1 viral loads were quantified on study Days 1, 8, 21, 29 and 43 by real-time reverse transcriptase polymerase chain reaction (RealTime HIV-1 assay; Abbott Diagnostics, IL, USA). $CD4^+$ T cell counts, and the analysis of CD38 expression on $CD8^+$ T cells was performed in HIV-positive subjects only on study Days 1, 8, 21,

29, and 43 by flow cytometry (FACSCalibur, Becton Dickinson) using Cellquest software. Peripheral Blood Mononuclear Cells (PBMCs) were fixed (FACS Lysing Solution, BD Bioscience), permeabilized (FACS Permeabilization Buffer, BD Bioscience), and stained with anti-CD4:FITC, anti-CD38:PE, and anti-CD8:PerCP monoclonal antibodies (all Pharmingen).

2.7 Statistical analysis

Statistical analyses were performed within and across vaccine groups. Safety results were evaluated descriptively. Immunogenicity endpoints were evaluated based on criteria for pandemic influenza vaccines established by the EU Committee for Medicinal Products for Human Use (CHMP): the percentage of subjects achieving seroconversion for HI antibody should be $\geqslant$40%; geometric mean ratios (GMRs) should be >2.5; and to achieve seroprotection, the percentage of subjects achieving an HI antibody titre $\geqslant$40% should be >70%. Two-sided 95% CIs were calculated according to the Clopper Pearson method. Immunogenicity results were logarithmically transformed ($base_{10}$) prior to analysis and responses were adjusted for baseline antibody status.

3. Results

Of the total participants enrolled, 90% and 91% of subjects in the aH1N1pnd and TIV vaccination groups completed the study on Day 43, respectively. The primary reasons for subjects not completing the study were their withdrawal of consent and being lost to follow-up. Subject disposition and study design are illustrated in Fig. 1. The age and body mass index of subjects in the aH1N1pnd and TIV vaccination groups and between HIV infected and non-infected were similar. The majority of subjects were Caucasian. Study populations demographics are presented in Table 1. Full Analysis Set (FAS) immunogenicity data are reported throughout.

3.1 Immunogenicity

Baseline HI geometric mean antibody titres (GMTs) were low in all four vaccination groups, ranging from 12 to 18 (Table 2). Three weeks after the administration of the first dose of either vaccine (Day 22), GMTs, the proportion of subjects achieving a seroprotective titre and those who had seroconverted were higher in the HIV-negative compared with the HIV-positive cohort (Table 2, Fig. 2). A second vaccine dose did not restore GMTs of HIV-infected subjects to the level of those seen in HIV-negative subjects. However, after two doses of the adjuvanted vaccine, 100% of HIV-infected subjects achieved seroprotective antibody titres of 40 or higher, as did all HIV uninfected subjects, while a lower proportion (90%) of HIV-infected subjects receiving unadjuvanted vaccine were seroprotected after two doses.

After the first dose of the MF59-adjuvanted vaccine, a greater proportion of HIV-infected subjects seroconverted (79%) compared to unadjuvanted vaccine recipients (68%). Similarly, in HIV-negative subjects, 85% of adjuvanted vaccine recipients seroconverted, versus 76% of those receiving unadjuvanted vaccine. The adjuvanted vaccine also provided a higher level of seroprotection after one dose in HIV-infected subjects (93%) than unadjuvanted vaccine (87%). All three CHMP licensure criteria were met by HIV-positive and HIV-negative cohorts in response to two doses of either aH1N1pnd or TIV.

3.2 HIV viral loads, $CD4^+$ T cell counts, and CD38 expression on $CD8^+$ T cells

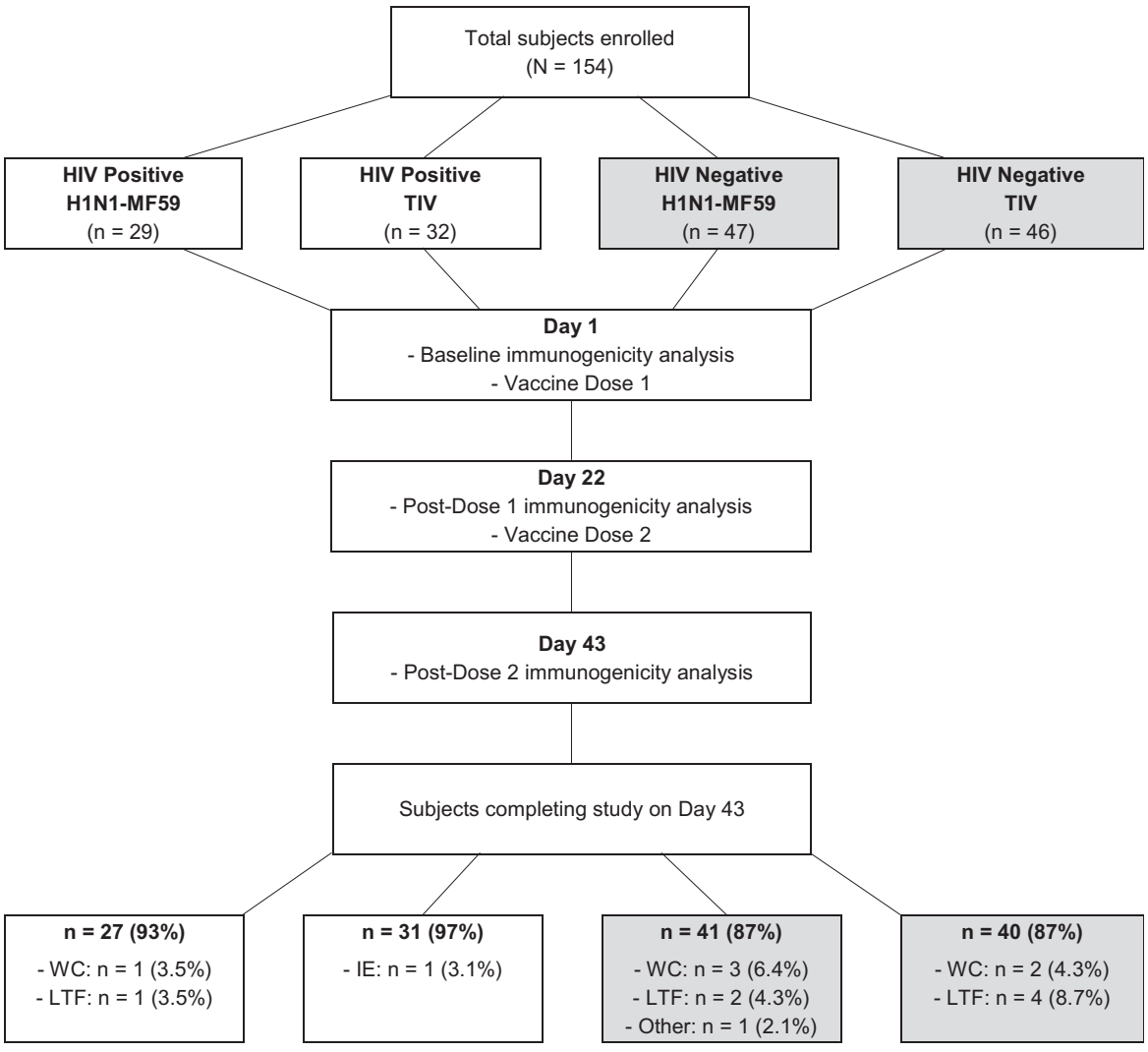
In the HIV-positive study groups receiving aH1N1pnd and TIV, HIV-1 viral loads remained below the limit of detection (<40 copies/mL) over the entire six-week study period. $CD4^+$ T cell counts in HIV-positive subjects receiving aH1N1pnd and TIV were performed on study Days 1, 8, 22, 29 and 43 (Fig. 3). Vaccination with aH1N1pnd or TIV had no effect on $CD4^+$ T cell counts relative to baseline values, with similar cell numbers observed following vaccination with first and second doses of adjuvanted and unadjuvanted vaccine. In the aH1N1pnd study group, mean $CD4^+$ T cell counts ranged from 562 cells/mm^3 on Day 1 to 582 cells/mm^3 on Day 43. In subjects receiving TIV, mean cell counts ranged from 527 cells/mm^3 on Day 1 to 609 cells/mm^3 on Day 43. As a marker of inflammation and immune activation, levels of CD38 expression on $CD8^+$ T cells were assessed in HIV-positive subjects by flow cytometry (Fig. 4). Vaccination with aH1N1pnd or TIV had no affect on levels of $CD8^+$ T cell CD38 expression relative to baseline values. Similar levels of CD38 were detected following vaccination with first and second doses of adjuvanted and unadjuvanted vaccines. Over the course of the study, the mean fluorescence intensity (MFI) of cell surface CD38 expression ranged from 102 (Day 1) to 163 (Day 43) in the aH1N1pnd group, and from 82 (Day 1) to 171 (Day 43) in the TIV group.

3.3 Safety

The percentages of subjects with treatment emergent AEs were similar in the HIV-positive and HIV-negative cohorts. The percentages of subjects with AEs were slightly higher in the adjuvanted (93.4%) compared with the unadjuvanted (88.3%) vaccine groups (Table 3). The most frequent local reaction was mild to moderate pain at the site of injection, experienced by similar percentages of HIV-positive subjects in the aH1N1pnd (55.2%) and TIV (56.3%) vaccination groups. In the HIV-negative cohort, 66% and 62% of subjects reported pain in aH1N1pnd and TIV groups, respectively. Overall, there was a trend towards less reactogenicity after the second vaccine dose. The majority of solicited local and systemic reactions were of mild to moderate severity, with 7.2% of both HIV-positive and HIV-negative subjects experiencing severe reactions across first and second doses. No cases of fever (>37.5 °C) or AEs of special interest occurred within any vaccination group during the study. Non-vaccine-related SAEs were experienced by one HIV positive subject in the aH1N1pnd vaccination group, and one HIV-negative subjects receiving TIV. Two SAEs (gingival bleeding and panniculitis) considered to be at least possibly related to TIV occurred in one HIV-negative subject (Table 3). No subjects were withdrawn from the study due to AEs.

4 Discussion

This study was performed to determine whether MF59-adjuvanted, A/H1N1 pandemic vaccine was more immunogenic in HIV-positive subjects than unadjuvanted vaccine. Although a single dose of either adjuvanted or unadjuvanted vaccine was sufficient to meet all three CHMP licensure criteria in both HIV-positive and control HIV-negative cohorts, the adjuvanted vaccine led to seroconversion in ~10% more of both subjects in both groups compared to unadjuvanted vaccine. In addition, after the first dose seroprotection was achieved in 6% more of HIV-infected subjects who received the adjuvanted vaccine. Overall it was clear that the responses of HIV-positive subjects were reduced compared with those of HIV-negative controls. A second dose of either vaccine did not lead to significantly increased antibody titres in HIV-infected or non-infected subjects, and did not compensate for the difference between the responses of HIV-infected and healthy subjects. Both vaccine formulations were generally well tolerated, with similar safety profiles observed in HIV-positive and HIV-negative subjects. No increase in reactogenicity was observed in



WC, withdrew consent; LTF, lost to follow-up; IE, inappropriate enrolment

Fig. 1. Study design. A total of 61 HIV-positive and 93 HIV-negative control subjects were enrolled in the study. Subjects received two doses of either MF59-adjuvanted, A/H1N1 pandemic influenza vaccine (aH1N1pnd), or two doses of unadjuvanted, trivalent, seasonal influenza vaccine (TIV). Immunogenicity was assessed by haemagglutination inhibition (HI) assay three weeks after immunization.

Table 1
Baseline demographics of enrolled study populations.

	aH1N1pnd (n = 76)	TIV (n = 77)
Mean age (years, SD)	42.6 (9.1)	42.0 (10.4)
Males (%)	46	61
Mean weight (kg, SD)	71.4 (15.4)	72.9 (14.4)
Mean height (cm, SD)	165.5 (9.9)	168.9 (9.0)
Mean BMI (kg/m^2, SD)	25.8 (3.8)	25.5 (4.6)
Previous influenza vaccine (%)	27.6	33.8
Former smoker (%)	30.3	24.7
Current smoker (%)	17.1	18.2
Caucasian (%)	75.0	83.1
Black (%)	6.6	6.5
Asian (%)	2.6	3.9
Mixed race (%)	15.8	6.5

BMI, body mass index.

response to the MF59-adjuvanted vaccine. We, however, recognize that the open label nature of the study and the absence of a control group may have influenced the description of symptoms related to the safety profile.

In general, the administration of a second dose of unadjuvanted pandemic or seasonal vaccine in HIV positive adults has not been successful in overcoming poor responses to the initial dose, although exceptions have been reported [17–19]. As reported in previous studies, we found a moderate increase in antibody levels following a second dose of MF59-adjuvanted A/H1N1 pandemic vaccine [12,20]. Some, but not all studies, have described similar results for AS03-adjuvanted vaccine [9,19,21]. Interestingly, doubling the antigen content of unadjuvanted subunit pandemic vaccine, from 15 to 30 μg, provided significantly better responses in HIV-infected subjects, although, their responses still fell below those of normal subjects [22]. Seroprotection rates have been reported to decline more rapidly in HIV-positive than HIV-negative individuals receiving unadjuvanted pandemic vaccine [10,20]. In studies of antibody persistence following adjuvanted and unadjuvanted A/H1N1 and A/H5N1 vaccination in healthy subjects, antibody titres to the adjuvanted formulations were maintained at higher levels up to one year later [23–28]. These data and other studies [13,18,29–31] suggest that an adjuvant may help maintain protection during the course of extended transmission during outbreaks.

Table 2
Immunogenicity analysis (95% CI) by haemagglutination inhibition (HI) assay against the A/California/7/2009 (H1N1) vaccine strain at baseline (Day 1), and three weeks after the administration of first (Day 22) and second (Day 43) vaccine doses.

	HIV-positive		HIV-negative	
	aH1N1pnd	TIV	aH1N1pnd	TIV
	Geometric mean titre			
Day 1	12 (7–21) (n = 29)	17 (10–29) (n = 32)	13 (8–21) (n = 47)	18 (11–29) (n = 44)
Day 22	288 (152–547) (n = 28)	415 (226–763) (n = 31)	682 (476–975) (n = 47)	826 (570–1196) (n = 45)
Day 43	410 (240–700) (n = 28)	370 (223–615) (n = 31)	785 (572–1076) (n = 42)	696 (509–951) (n = 44)
	Geometric mean ratio			
Day 22:Day 1	22 (10–47) (n = 28)	25 (12–50) (n = 31)	52 (30–90) (n = 47)	47 (27–83) (n = 44)
Day 43:Day 1	31 (16–60) (n = 28)	22 (12–41) (n = 31)	59 (34–101) (n = 42)	39 (23–66) (n = 43)
	% Seroconversion or ⩾4-fold increase			
Day 22	79 (59–92) (n = 28)	68 (49–83) (n = 31)	85 (72–94) (n = 47)	76 (61–87) (n = 45)
Day 43	79 (59–92) (n = 28)	71 (52–86) (n = 31)	86 (72–95) (n = 42)	84 (70–93) (n = 44)

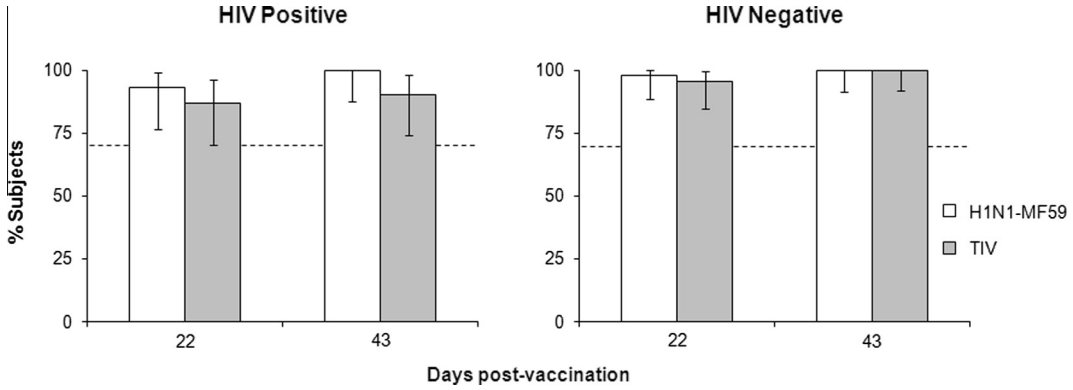


Fig. 2. Seroprotection rates among HIV-positive and HIV-negative subjects three weeks after first (Day 22) and second (Day 43) vaccine doses. Broken line represents the CHMP licensure criterion for seroprotection.

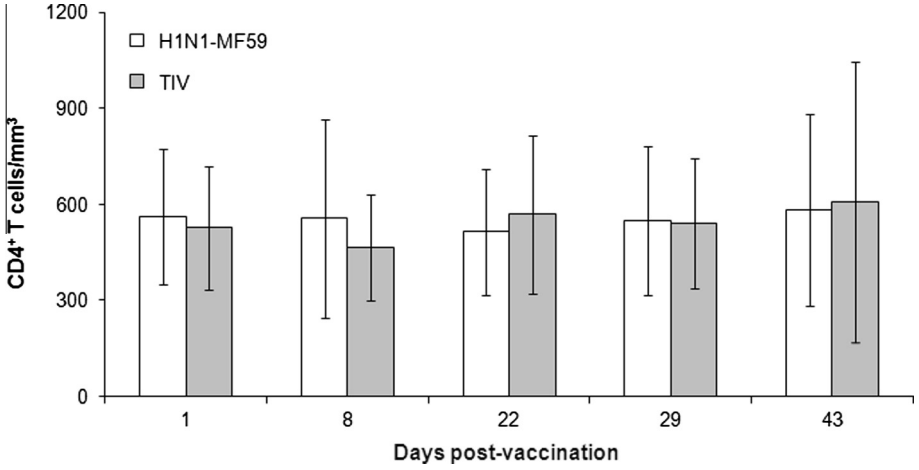


Fig. 3. Mean (±SD) $CD4^+$ T cell counts in HIV-positive subjects at baseline (Day 1), one (Day 8) and three (Day 22) weeks after first, and one (Day 29) and three (Day 43) weeks after second aH1N1pnd or TIV vaccine doses.

One of the limitations of this study was the use of unadjuvanted TIV containing the A/H1N1 pandemic strain rather than a unadjuvanted, monovalent, pandemic vaccine as a control, which was unavailable when the study commenced. Although the TIV comparator contained the same pandemic viral strain with the usual quantity (15 μg) of viral haemagglutinin, the antigen was derived from split virions, while the monovalent pandemic vaccine contained purified haemagglutinin subunits. As responses to split

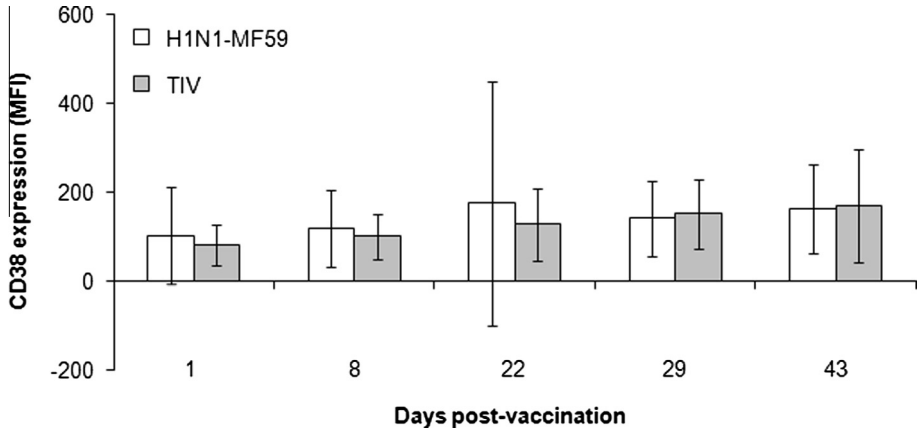


Fig. 4. Mean fluorescence intensity (MFI) of CD38 expression (±SD) on $CD8^+$ T cells in HIV-positive subjects, as measured by flow cytometry at baseline (Day 1), one (Day 8) and three (Day 22) weeks after first, and one (Day 29) and three (Day 43) weeks after second aH1N1pnd or TIV vaccine doses.

Table 3
Percentages of subjects experiencing adverse events throughout the entire study period (Day 1–43).

	HIV-positive		HIV-negative	
	aH1N1pnd (n = 29)	TIV (n = 32)	aH1N1pnd (n = 47)	TIV (n = 45)
Adverse events (%)	93	88	94	89
Vaccine-related adverse events (%)	72	69	77	76
Severe adverse events (%)	7	6	9	7
Serious adverse events (%)	3	0	0	4

antigens are, in general, similar to or higher than those to subunit TIVs, differences between the responses to adjuvanted and unadjuvanted vaccines in the trial would have tended toward an underestimation of the adjuvant's effect. A limitation of many other trials of A/H1N1 pandemic vaccines was the potential for acquired pandemic infections to interfere with interpretation of the immune response to vaccination; this was unlikely in our study, which was completed in June while the peak of the pandemic in southern Brazil was in early August.

It is conceivable that vaccination could transactivate HIV and transiently increase the viral load [32,33] with the potential risk of antiretroviral resistance [34], which has not been observed in the current study. The expression of CD38 on the surface of $CD8^+$ T cells is indicative of cellular activation, and in HIV-positive individuals, correlates with plasma viremia and $CD4^+$ T cell numbers; levels of $CD8^+$ T cell CD38 expression are also reported to reliably predict the onset of acquired immunodeficiency syndrome (AIDS) – particularly in patients receiving antiretroviral therapy [35–41], blunted $CD4^+$ T cell recovery [33,42] and non-AIDS clinical outcomes [43]. Vaccination had no impact on levels of CD38 expression, or $CD4^+$ T cell numbers over the course of this study. The six-week observational period of this trial was insufficient to adequately monitor possible changes in $CD4^+$ T cells numbers and levels of CD38 expression resulting from vaccination. A prolonged investigation over twelve months is required to fully assess the effects of adjuvanted versus unadjuvanted vaccination on T lymphocyte numbers and phenotype. An extended study would also allow for comprehensive analysis of the possible benefits of MF59 adjuvant on long-term antibody persistence in immunosuppressed HIV-positive individuals. The relatively high $CD4^+$ T cell counts and low viral loads of the HIV-positive subjects enrolled in this trial suggest that these individuals were not immunocompromised and were essentially in good health. Therefore, HIV-positive subjects were able to respond well to the unadjuvanted vaccine. Further studies involving severely immunosuppressed subjects, with higher viral loads and lower $CD4^+$ T cell counts, are required to determine whether MF59-adjuvanted vaccine may be of significant benefit to immunosuppressed HIV-positive individuals.

In conclusion, well-controlled HIV-infected patients on retroviral therapy responded adequately but less well to the A/H1N1 pandemic vaccine than healthy control subjects, whether antigen was in the form of a routine, unadjuvanted, seasonal vaccine or in an MF59-adjuvanted, monovalent, A/H1N1 pandemic formulation. A second dose of either vaccine did not compensate for the relatively lower response in HIV positive subjects. All vaccinations were well tolerated and did not affect viral loads, $CD4^+$ T cell counts, or levels of CD38 expression on $CD8^+$ T cells.

Author contributions

All authors participated in the conception, design and implementation of this clinical trial. All authors were involved in the interpretation of analysed data and the decision to submit for publication.

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