Preparedness against an A/H5N1 influenza pandemic requires well-tolerated, effective vaccines which provide both vaccine strain-specific and heterologous, cross-clade protection. This study was conducted to assess the immunogenicity and safety profile of an MF59-adjuvanted, prepandemic influenza vaccine containing A/turkey/Turkey/01/2005 (H5N1) strain viral antigen. A total of 343 participants, 194 adults (18 to 60 years) and 149 elderly individuals (≥61 years), received two doses of the investigational vaccine given 3 weeks apart. Homologous and heterologous antibody responses were analyzed by hemagglutination inhibition (HI), single radial hemolysis (SRH), and microneutralization (MN) assays 3 weeks after administration of the first vaccine dose and 3 weeks and 6 months after the second dose. Immunogenicity was assessed according to European licensure criteria for pandemic influenza vaccines. After two vaccine doses, all three European licensure criteria were met for adult and elderly subjects against the homologous vaccine strain, A/turkey/Turkey/1/2005, when analyzed by HI and SRH assays. Cross-reactive antibody responses were observed by HI and SRH analyses against the heterologous H5N1 strains, A/Indonesia/5/2005 and A/Vietnam/1194/2004, in adult and elderly subjects. Solicited local and systemic reactions were mostly mild to moderate in severity and occurred less frequently in the elderly than in adult vaccinees. In both adult and elderly subjects, MF59-adjuvanted vaccine containing 7.5 μg of A/Turkey strain influenza virus antigen was highly immunogenic, well tolerated, and able to elicit cross-clade, heterologous antibody responses against A/Indonesia and A/Vietnam strains 6 weeks after the first vaccination.
2005 (H5N1) in adult and elderly subjects, according to European licensure criteria established by the European Committee for Medicinal Products for Human Use (CHMP) (10). The secondary objective of this study was the assessment of cross-reactive antibody responses.

**Vaccine.** One 0.5-ml dose of the investigational, inactivated, egg-derived, MF59-adjuvanted, preponderant vaccine contained 7.5 μg of A/turkey/Turkey/1/2005 (H5N1; clade 2.2.1) influenza virus strain hemagglutinin surface antigen and a standard dose (9.75-mg squalene) of MF59 adjuvant, as found in the European licensed seasonal influenza vaccine Fluad (Novartis Vaccines and Diagnostics). Vaccine was supplied in prefilled monodose (0.5 ml) syringes and administered in the deltoid muscle of the nondominant arm.

**Immunogenicity assessment.** Blood samples (~20 ml per sample) were collected for immunogenicity analysis at baseline (day 1), 3 weeks after administration of the first vaccine dose (day 22), and 3 weeks (day 43) and approximately 6 months (day 202) after administration of the second dose. Serum aliquots were stored at −18°C and shipped to the Novartis Vaccines Clinical Serology Laboratory in Marburg, Germany, and the Department of Physiopathology, Experimental Medicine and Molecular Epidemiology, University of Siena, Siena, Italy. Antibody levels were determined by hemagglutination inhibition (HI) and microneutralization (MN) assays in Marburg and by single radial hemolysis (SRH) in Siena. The HI assay was performed using horse erythrocytes and based on methods described by Stephenson and colleagues (29); HI titer is expressed as the reciprocal of the highest dilution at which hemagglutination was totally inhibited. MN assays were performed according to methods described by Nicholson and colleagues (23); serial dilutions of serum started at 10; the reciprocals of 2-fold dilutions that achieved ≥50% neutralization of viral growth were considered to be a positive result. The protocol for SRH was based on methods described by Schild and colleagues (27). Seroconversion in the HI assay was defined as a negative prevaccination antibody titer of <10 to a positive postvaccination titer of ≥40; in the MN assay, a titer of <20 becoming ≥40; and in the SRH assay, an area of ≤4 mm² becoming ≥25 mm². A significant increase in antibody titer in HI and MN assays was defined as a ≥4-fold increase and in the SRH assay as a ≥50% increase in area. HI and MN titers below the detection limits of 10 and 20, respectively, were arbitrarily assigned to half that limit for the purpose of analysis. SRH areas below the detection limit were given a value of 4 mm². Homologous antibody titers were measured by HI, SRH, and MN assays against the vaccine antigen strain A/turkey/Turkey/1/2005. Cross-reactive antibody titers were measured by HI, SRH, and MN assays against the heterologous H5N1 strains A/Vietnam/1194/2004 (clade 1) and A/Indonesia/5/2005 (clade 2.1).

**Safety assessment.** Subjects were observed for a minimum of 30 min after vaccine administration to monitor for possible immediate reactions. Vaccinees were provided with diary cards and asked to record any specified local or systemic reaction occurring within 1 week of vaccination. Solicited local reactions were pain at the site of injection, erythema, induration, swelling, and ecchymosis. Solicited systemic reactions were fever (≥38°C), chills, malaise, myalgia, arthralgia, headache, sweating, nausea, fatigue, vomiting, and diarrhea. Unsolicited adverse events (AEs) were recorded for 3 weeks after each vaccination. All serious adverse events (SAEs) and AEs requiring the attention of a physician or leading to withdrawal were recorded throughout the study period (days 1 to 202). The investigator used a standard scale to grade AEs, which were defined as mild, moderate, or severe if resulting in no limitation of, some limitation of, or an inability to perform normal daily activities, respectively.

**Statistical analyses.** There was no formal statistical hypothesis tested. Immunogenicity endpoints were based on the following HI (CHMP) licensure criteria: the number of subjects achieving seroconversion or significantly increased antibody titers should be >40% and >30% for adult and elderly subjects, respectively; geometric mean ratios (GMRs) should be >2.5 for adults and >2.0 for the elderly; and for seroprotection, the proportion of subjects achieving an HI titer of ≥40 or an SRH area of ≥25 mm² should be >70% and >60% for adults and the elderly, respectively.

**RESULTS**
A total of 343 healthy volunteers participated, 194 adults (18 to 60 years) and 149 elderly (≥61 years) subjects, of whom 99% completed the study (Fig. 1). All participants were Caucasian. Adult

![Diagram](https://cvi.asm.org/Downloaded from http://cvi.asm.org/ on July 20, 2018 by guest)
and elderly subject groups were of similar weights and heights. More elderly (43%) than adult (20%) subjects had previously received influenza vaccine (Table 1). The Full Analysis (FAS) and Per Protocol (PPS) data sets differed by <10%; PPS immunogenicity data are reported throughout.

**Immunogenicity analysis.** Antibody responses against the vaccine strain antigen (A/turkey/Turkey/1/2005) are shown in Table 2. After the first vaccine dose (day 22), there were fewer licensure criteria met in either group in any of the three assays—specifically, elderly subjects met the licensure criterion for seroconversion by HI assay (32%); adults achieved GMRs of 2.6 and 2.5 in HI and SRH, respectively; and elderly subjects had an HI GMR of 2.8. After the second vaccine dose (day 43), adult and elderly groups met all three CHMP licensure criteria. Adult and elderly groups achieved HI seroconversion rates of 69% and 62% and SRH seroconversion rates of 85% and 70%, respectively. The CHMP criterion for GMR was met in both age groups by HI and SRH analyses. Adult subjects met the criterion for seroconversion by SRH alone (91%), while both HI (64%) and SRH (82%) assays found elderly subjects to be seroprotected. These data were supported by MN analyses throughout.

**Solicited local and systemic reactions.** Solicited local and systemic reactions occurred during the 2 weeks of vaccination and were summarized in Table 5. The majority of solicited reactions were mild to moderate in severity and were more frequent in adult than elderly subjects. More local and systemic reactions were reported after the second (55%) than the first (41%) dose in adults and in elderly subjects (first, 36%; second, 26%). The most frequently reported local reaction in adults was pain, experienced by 43% and 31% of subjects after first and second doses, respectively, of which only one case, after the first dose in an elderly subject, was described as severe. Myalgia was the most common systemic reaction in adults, reported by...
13% and 7% of subjects after first and second doses, respectively. Only 1% of adults experienced fever (≥38°C), and no cases of severe fever (≥40°C) were reported. Vaccine-related AEs were experienced by 2% of adult subjects after both first and second doses; all were of mild to moderate severity and usually local or systemic reactions, such as pain. No vaccine-related AEs were reported after day 43. One adult subject withdrew consent after receiving the first vaccine dose; no specific reason for this decision was given.

**DISCUSSION**

The pandemic threat posed by the A/H5N1 (avian) influenza virus is significant, and ongoing efforts to protect the human population against the possible emergence of a highly virulent strain capable of human-to-human transmission are essential. A recent study by Imai et al. investigated the molecular features which could render H5 viruses transmissible in mammals; animal studies indicate that genetic reassortment involving as few as four mutations in the hemagglutinin surface protein are sufficient for efficient viral transmission to occur (15). These findings emphasize the need to prepare for potential avian influenza pandemics. Active priming in those who have not encountered the A/H5N1 influenza virus serves to equip the individual with A/H5N1-specific memory lymphocyte populations and decrease the number of vaccinations required in the event of a pandemic (16, 26). This clinical trial evaluated the immunogenicity and safety profiles of

### TABLE 3 Immunogenicity analysis against the heterologous strain A/Indonesia/5/2005 (H5N1)*

| Assay (no. of adults/no. of elderly) | Parameter | Day(s) | Adults | Elderly |
|-------------------------------------|-----------|--------|--------|---------|
| HI (194/148)                         | GMT       | 1      | 5.4 (5.1–5.6) | 5.4 (5.1–5.6) |
|                                     |           | 43     | 25 (20–32) | 14 (12–18) |
|                                     |           | 202    | 7.1 (6.3–7.9) | 6.9 (6.3–7.7) |
| GMR                                | 43/1      | 4.7 (3.7–5.9) | 2.7 (2.2–3.3) |
|                                     | 202/1     | 1.3 (1.2–1.5) | 1.3 (1.2–1.4) |
| SP (%)                             | 1         | 0.5 (0–2.9) | 0 (0–2.5) |
|                                     | 43        | 50 (43–57) | 34 (26–42) |
|                                     | 202       | 11 (6.9–16) | 4.1 (1.5–8.6) |
| SC (%)                             | 43        | 49 (42–56) | 32 (25–41) |
|                                     | 202       | 8.3 (4.8–13) | 4.1 (1.5–8.6) |
| SRH (187/143)                      | GMA       | 1      | 6.2 (5.5–6.9) | 6.6 (5.8–7.6) |
|                                     |           | 43     | 38 (35–43) | 26 (23–30) |
|                                     |           | 202    | 11 (9.7–12) | 11 (9.2–12) |
| GMR                                | 43/1      | 6.2 (5.4–7.2) | 3.9 (3.3–4.5) |
|                                     | 202/1     | 1.8 (1.6–2.0) | 1.6 (1.4–1.8) |
| SP (%)                             | 1         | 11 (7–16) | 9 (5–15) |
|                                     | 43        | 83 (77–88) | 61 (52–69) |
|                                     | 202       | 22 (16–28) | 21 (15–29) |
| SC (%)                             | 43        | 79 (72–85) | 64 (56–73) |
|                                     | 202       | 19 (14–26) | 18 (12–25) |

| MN (194/148)                       | GMT       | 1      | 5.0 (5.0–5.1) | 5.0 (5.0–5.0) |
|                                     |           | 43     | 24 (20–28) | 11 (9–13) |
|                                     |           | 202    | 7.2 (6.5–7.9) | 6.0 (5.5–6.4) |
| GMR                                | 43/1      | 4.7 (3.9–5.6) | 2.2 (1.9–2.6) |
|                                     | 202/1     | 1.4 (1.3–1.6) | 1.2 (1.1–1.3) |
| Titer ≥40 (%)                      | 1         | 0 (0–2.0) | 0 (0–2.0) |
|                                     | 43        | 38 (31–45) | 14 (8–20) |
|                                     | 202       | 4.0 (1.0–7.0) | 1.0 (0–5.0) |
| ≥4-fold increase (%)               | 43        | 38 (30–65) | 30 (23–38) |
|                                     | 202       | 11 (7.0–16) | 5.0 (2.0–10) |

* Bold text indicates that CHMP licensure criteria were met. SP, seroprotection (HI titer of ≥40; SRH area of ≥25 mm²); SC, seroconversion (HI titer of <10 to ≥40; SRH area of ≤54 mm² to ≥25 mm²); or significant increase (≥4-fold increase in HI titer; ≥50% increase in SRH area); CI, confidence interval.

### TABLE 4 Immunogenicity analysis against the heterologous strain A/Vietnam/1194/2004 (H5N1)*

| Assay (no. of adults/no. of elderly) | Parameter | Day(s) | Adults | Elderly |
|-------------------------------------|-----------|--------|--------|---------|
| HI (194/148)                         | GMT       | 1      | 5.9 (5.5–6.5) | 6.9 (6.0–7.9) |
|                                     |           | 43     | 25 (20–32) | 19 (15–25) |
|                                     |           | 202    | 9.2 (7.9–11) | 11 (8.9–13) |
| GMR                                | 43/1      | 4.3 (3.4–5.4) | 2.8 (2.2–3.6) |
|                                     | 202/1     | 1.6 (1.4–1.8) | 1.5 (1.4–1.9) |
| SP (%)                             | 1         | 3.1 (1–6.6) | 5.4 (2.4–10) |
|                                     | 43        | 47 (40–55) | 39 (31–48) |
|                                     | 202       | 18 (13–24) | 17 (11–24) |
| SC (%)                             | 43        | 44 (37–51) | 34 (26–42) |
|                                     | 202       | 12 (7–17) | 12 (6–18) |

| SRH (187/143)                      | GMA       | 1      | 5.1 (4.8–5.5) | 5.3 (4.9–5.9) |
|                                     |           | 43     | 23 (20–26) | 16 (14–19) |
|                                     |           | 202    | 7.3 (6.3–8.0) | 8.2 (7.2–9.4) |
| GMR                                | 43/1      | 4.5 (3.9–5.1) | 3.0 (2.6–3.6) |
|                                     | 202/1     | 1.4 (1.3–1.6) | 1.5 (1.4–1.7) |
| SP (%)                             | 1         | 4.0 (2–8.0) | 5.0 (2–10) |
|                                     | 43        | 62 (54–69) | 45 (37–54) |
|                                     | 202       | 10 (6–15) | 14 (9–21) |
| SC (%)                             | 43        | 60 (53–68) | 44 (35–53) |
|                                     | 202       | 10 (6–15) | 14 (9–21) |

| MN (194/148)                       | GMT       | 1      | 5.0 (5.0–5.1) | 5.2 (5.0–5.4) |
|                                     |           | 43     | 9.4 (8.2–11) | 6.9 (5.9–8.0) |
|                                     |           | 202    | 5.7 (5.3–6.0) | 6.3 (5.6–7.2) |
| GMR                                | 43/1      | 1.9 (1.6–2.1) | 1.3 (1.2–1.5) |
|                                     | 202/1     | 1.1 (1–1.2) | 1.2 (1–1.4) |
| Titer ≥40 (%)                      | 1         | 0 (0–2.0) | 1.0 (0–4.0) |
|                                     | 43        | 10 (6–16) | 6.0 (3–11) |
|                                     | 202       | 1.0 (0–4.0) | 4.0 (2–9.0) |
| ≥4-fold increase (%)               | 43        | 19 (13–25) | 7.0 (4–13) |
|                                     | 202       | 4.0 (2–8.0) | 18 (4–14) |

* Bold text indicates that CHMP licensure criteria were met. SP, seroprotection (HI titer of ≥40; SRH area of ≥25 mm²); SC, seroconversion (HI titer of <10 to ≥40; SRH area of ≤54 mm² to ≥25 mm²); or significant increase (≥4-fold increase in HI titer; ≥50% increase in SRH area); CI, confidence interval.
an MF59-adjuvanted, pandemic influenza vaccine containing clade 2 A/H5N1 viral strain antigen (A/turkey/Turkey/01/2005). Vaccine antigen-specific and cross-reactive antibody responses were assessed. The results of this study support previously published data from clinical trials of the investigational vaccine (12).

Antibody responses against the vaccine antigen strain were sufficient to meet the European licensure criteria after two doses in both adult and elderly subjects. The results of this study are consistent with similar trials of MF59-adjuvanted A/H5N1 vaccine containing A/Vietnam/1194/2004 strain antigen, which also found that adult and elderly subjects require two vaccine doses (2, 4). Modeling studies suggest that A/H5N1 vaccines conferring even moderate heterologous protection can substantially mitigate the impact of a pandemic (11). The levels of cross-reactive antibody production observed throughout this study were uncharacteristically low when measured by MN assay. However, cross-reactive data generated by HI and SRH assays demonstrate that the MF59-adjuvanted vaccine induced seroprotective heterologous antibody titers. These data are consistent with previous clinical trials which also show that MF59 enhances the production of cross-reactive antibodies (2, 12, 13, 18–20). A study by Galli et al. showed that MF59-adjuvanted vaccine induced a 3-fold expansion in A/H5N1-specific CD4+ T cells able to react with clade 0-like and clade 2 A/H5N1 proteins, confirming that MF59 promotes the development of broadly cross-reactive T cells and thereby heterologous B cell antibody responses (14).

MF59 has a well-established safety record in vaccinees of all ages (2–5, 21, 25, 30, 31). Use of a similar oil-in-water emulsion adjuvant in A/H1N1 pandemic vaccines has been associated with narcolepsy in young children (35), but a recent investigation found no evidence of an association between MF59 adjuvant and increased risk of narcolepsy (6, 31). The investigational vaccine was generally well tolerated by adult and elderly subjects, with the majority of reactions being of mild to moderate severity and rapidly resolved. The tolerability profiles generated during this study were similar to those observed in other trials of MF59-adjuvanted A/H5N1 vaccines in adults and the elderly (2, 4, 21). MF59 has been shown to promote long-term antibody persistence (14, 32), an element essential to successful pandemic vaccination strategy. Immunogenicity analysis 1 year after vaccination would be beneficial in order to evaluate MF59-enhanced long-term antibody persistence. All study participants were deemed to be healthy on enrollment; therefore, the findings of this investigation should not be applied to the immunosuppressed or those affected by chronic conditions.

The MF59-adjuvanted, pandemic, investigational vaccine was shown to be well tolerated and adequately immunogenic in terms of both vaccine antigen-specific and cross-reactive antibody responses. These data and a previous report (12) demonstrate the investigational vaccine, containing 7.5 µg of clade 2, H5N1, A/turkey/Turkey/II/2005 strain influenza antigen per dose, to be suitable for pre-pandemic use in adult and elderly populations.

TABLE 5 Percentages of subjects experiencing mild to moderate (and severe) solicited local and systemic reactions within 1 week of vaccination

| Reaction         | Adults 1st dose | Adults 2nd dose | Elderly 1st dose | Elderly 2nd dose |
|------------------|----------------|----------------|-----------------|-----------------|
| Erythema         | 12 (0)         | 14 (1)         | 18 (1)          | 17 (0)          |
| Induration       | 7 (0)          | 6 (1)          | 7 (0)           | 7 (0)           |
| Swelling         | 9 (0)          | 9 (1)          | 12 (1)          | 7 (0)           |
| Ecchymosis       | 3 (0)          | 2 (0)          | 3 (0)           | 1 (0)           |
| Pain             | 43 (0)         | 31 (0)         | 14 (1)          | 14 (0)          |

% of subjects with mild to moderate (% with severe) reactions

Local reactions

Systemic reactions

Chills
Malaise
Myalgia
Arthralgia
Headache
Sweating
Nausea
Vomiting
Diarrhea
Fatigue
Fever (≥38°C)

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