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A randomized clinical trial to identify the optimal antigen and MF59® adjuvant dose of a monovalent A/H1N1 pandemic influenza vaccine in healthy adult and elderly subjects

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ABSTRACT

Background: Vaccines against pandemic A/H1N1 influenza are required to protect the entire population. This dose range study aimed to identify priming antigen and adjuvant doses resulting in optimal levels of antibody-mediated protection after primary and one-year booster immunizations.
Methods: This randomised trial enrolled 410 healthy adult (18–60 years) and 251 healthy elderly (>60 years) participants. Subjects received vaccine containing either 3.75 μg or 7.5 μg antigen, adjuvanted with half the standard dose, or a standard dose of MF59® (Novartis Vaccines) adjuvant, respectively. An additional adult cohort received non-adjuvanted vaccine containing 15 μg antigen. Two doses of investigational vaccine were administered three weeks apart, followed by a single booster dose of adjuvanted seasonal influenza vaccine one year after priming. Immunogenicity was assessed by haemagglutination inhibition and microneutralization assays pre- and post-immunization, the safety profile of each vaccine was also evaluated.
Results: All of the vaccine formulations investigated were highly immunogenic and well tolerated in both adult and elderly subjects. The 7.5 μg formulation induced the highest antibody titres after primary and booster immunizations, and resulted in better long-term antibody persistence, in both age groups. Assessment according to European licensure criteria for influenza vaccines concluded that single adjuvanted priming doses containing 3.75 μg and 7.5 μg antigen were optimal for the adult and elderly populations, respectively.
Conclusions: These data demonstrate that one priming dose of MF59-adjuvanted A/H1N1 vaccine provided healthy adult (3.75 μg or 7.5 μg formulations) and healthy elderly (7.5 μg formulation) individuals with adequate levels of seroprotection. Booster administration after two priming doses of either vaccine formulation resulted in the rapid development of seroprotective antibody titres.
Trial registration: www.clinicaltrials.gov (NCT00971906).

1. Introduction

The rapid global spread of novel swine origin influenza A/H1N1 resulted in the World Health Organization declaring a phase six pandemic in June 2009 [1]. Mass vaccination is the most effective method of preventing disease in the individual and limiting viral transmission within communities. Health authorities and vaccine manufactures have focused on the development of effective vaccines based on the A/California/7/2009 viral strain; various adjuvanted and non-adjuvanted vaccine formulations have been produced using both traditional and novel methods [2].

Any candidate vaccine must: provide adequate levels of seroprotection in the entire population, including the elderly and

Abbreviations: HI, haemagglutination inhibition; MN, microneutralization; CHMP, European committee for medicinal products for human use; AE, adverse event; SAE, serious adverse event; GMR, geometric mean ratio; GMT, geometric mean titre; ANOVA, analysis of variance; SD, standard deviation; CI, confidence interval.

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immunocompromised; induce a degree of heterologous immunity, sufficient to provide protection should the pandemic viral strain differ considerably from the vaccine antigen, or mutate during the later stages of a pandemic; result in clinically significant levels of long-term antibody persistence; and use a minimal quantity of antigen per dose, in order to ensure the widest possible coverage despite limited global manufacturing capacity [3]. Vaccine adjuvants are the optimal method to ensure these demands are met. The most promising adjuvants to date are the oil-in-water emulsions, MF59® (Novartis Vaccines), AS03® (GlaxoSmithKline Biologicals), and AF03® (Sanofi Pasteur).

Several controlled clinical trials have demonstrated that MF59 adjuvant promotes all the qualities required of a safe [4–12] and immunogenically effective A/H1N1 pandemic vaccine [11,13–17]. In addition to optimizing antibody responses to immunization with much reduced antigen content [18,19], MF59 has been shown to increase antibody titres to seroprotective levels in the elderly and immunocompromised [20], result in improved levels of long-term antibody persistence [21], and provide heterologous protection by promoting the production of cross-reactive antibodies [22–27].

This randomized, multi-centre clinical trial evaluated the immunogenicity of three different A/H1N1 vaccine formulations in order to identify the quantities of priming antigen and adjuvant required to elicit optimal levels of seroprotection after primary and one-year booster immunizations in healthy adult and elderly subjects. Levels of long-term antibody persistence and the safety profile of each vaccine were also assessed.

2. Methods

2.1. Study design and objectives

This randomised, single-blind, multi-centre study was performed across eight sites in Germany, two sites in Belgium, and two sites in Switzerland between August 2009 and April 2011. The protocol was approved by the Ethics Review Committee of each participating centre, and the studies were conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from each participant before enrolment. The study was registered with the National Institutes of Health database, ClinicalTrials.gov (NCT00971906). The primary objective of this study was the identification of antigen and adjuvant doses resulting in the highest levels of immunogenicity and best safety profile following primary immunization. The secondary objectives were the identification of priming antigen and adjuvant doses resulting in the highest levels of long-term antibody persistence, and highest antibody concentrations in response to booster immunization. Participants received two primary doses of monovalent A/H1N1 vaccine administered three weeks apart (Day 1 and Day 22), followed approximately one year (Day 366) later by a single booster dose of MF59-adjuvanted trivalent seasonal influenza vaccine. Sera were collected for immunogenicity analysis at baseline (Day 1), three weeks after each primary dose (Days 22 and 43), one year after primary immunization (Day 366), and three weeks after booster immunization (Day 387). Subject disposition and study design are illustrated in Fig. 1.

2.2. Subjects

A total of 661 healthy adult (18–60 years) and healthy elderly (>60 years) volunteers were enrolled. The main exclusion criteria were: receipt of adjuvanted influenza vaccine, or influenza disease less than three months before study Day 1; any current chronic or progressive disease (including neoplasm, insulin dependent diabetes, cardiac, renal, or hepatic disease); receipt of any other vaccines less than four weeks before enrolment, with the exception of non-adjuvanted seasonal influenza vaccine; any history of serious reaction to vaccination, or hypersensitivity to influenza or chicken proteins; receipt of any other investigational agent less than four weeks prior to enrolment; an impaired or altered immune system; a history of progressive or severe neurological disorders; female subjects either pregnant, breastfeeding, or refusing to use an acceptable method of birth control for three weeks following vaccination; and receipt of any blood products less than twelve weeks before enrolment.

2.3. Vaccines

The investigational, egg-derived, monovalent, MF59-adjuvanted, pandemic influenza vaccine, Focetria® (Novartis Vaccines), contained haemagglutinin and neuraminidase surface antigens derived from the A/California/7/2009 (H1N1) influenza strain. The vaccine seed A/H1N1 virus was prepared from the reassortant virus, NYMC X-179A (New York Medical College, New York, USA), generated from the A/California/7/2009 strain, as recommended by the World Health Organization [28]. Adult subjects were assigned in equal numbers to one of three immunization groups to receive vaccine either containing 3.75 μg antigen with half the standard dose of MF59 adjuvant (3.75-Half MF59, Lot Z56P18N1), 7.5 μg antigen with a standard dose of MF59 (7.5-Full MF59, Lot Z56P18N1), or 15 μg antigen with no MF59 (15-No MF59, Lot Z53P22N1). Elderly subjects were assigned (1:1) to receive either the 3.75-Half MF59 or 7.5-Full MF59 vaccine formulations. A standard dose of MF59, as used in the commercial seasonal influenza vaccine, Fluad®, contains 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 booster dose of the seasonal, MF59-adjuvanted, trivalent influenza vaccine (ATIV), Fluad (Novartis Vaccines), contained a standard dose of MF59, and 15 μg of each of the World Health Organization reference strains recommended for the 2010/11 influenza season: A/California/7/2009 (H1N1); A/Perth/16/2009 (H3N2); and B/Brisbane/60/2008 (Lot 104002E/104601E/104601A). Thus, the A/H1N1 antigen strain contained in both priming and booster vaccines were identical. All vaccines were administered in the deltoid muscle of the non-dominant arm using a 23 gauge hypodermic needle. All vaccines were administered in a volume of 0.5 mL per dose, apart from the 3.75-Half MF59 formulation, which was administered in a volume of 0.25 mL per dose.

2.4. Immunogenicity analysis

Blood samples (~20 mL per sample) were obtained by venipuncture using vaccuumized collection tubes and centrifuged at $1500 \times g$ for 10 min; sera were stored at a temperature of −18 °C or below and shipped to the Novartis Vaccines Clinical Serology Laboratory in Marburg, Germany, where antibody responses were assessed by haemagglutination (HI) and MN (microneutralization) assays. The HI assay was based on the method of Stephenson and colleagues [29]; HI titre was expressed as the reciprocal of the highest dilution at which haemagglutination was totally inhibited. MN assays were performed according to a method described by Nicholson and colleagues [30]; serial dilutions of serum started at 1:20; the reciprocals of two-fold dilutions that achieved ≥ 50% neutralization of viral growth were considered a positive result. Seroconversion, as assessed by HI assay, was defined as a negative pre-vaccination antibody titre of <10 to a positive post-vaccination titre of ≥ 40, or a minimum four-fold increase where pre-vaccination titres were ≥10. HI titres below the detection limit of 10 were arbitrarily assigned to half that limit (1:5) for the