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Phase 2 Assessment of the Safety and Immunogenicity of Two Inactivated Pandemic Monovalent H1N1 Vaccines in Adults as a Component of the U.S. Pandemic Preparedness Plan in 2009

Wilbur H. Chen¹, Patricia L. Winokur^{2,3}, Kathryn M. Edwards⁴, Lisa A. Jackson⁵, Anna Wald⁶, Emmanuel B. Walter⁷, Diana L. Noah⁸, Mark Wolff⁹, and Karen L. Kotloff¹ on behalf of the Pandemic H1N1 Vaccine Adult Study Group

¹Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD

²University of Iowa Carver College of Medicine, Iowa City, IA

³Iowa City VA Health Care System, Iowa City, IA

⁴Vanderbilt Vaccine Research Program, Vanderbilt University School of Medicine, Nashville, TN

⁵Group Health Research Institute, Seattle, WA

⁶University of Washington School of Medicine, and Fred Hutchinson Cancer Research Center, Seattle, WA

⁷Duke Clinical Vaccine Unit, Duke University School of Medicine, Durham, NC

⁸Southern Research Institute, Birmingham, AL

⁹EMMES Corp, Rockville, MD

Abstract

Background—The influenza A/H1N1 pandemic in 2009 created an urgent need to develop vaccines for mass immunization. To guide decisions regarding the optimal immunization dosage and schedule for adults, we evaluated two monovalent, inactivated, unadjuvanted H1N1 influenza

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Corresponding author: Karen L. Kotloff, MD, Center for Vaccine Development, University of Maryland School of Medicine 685 W. Baltimore Street, Suite 480, Baltimore, MD 21201, Telephone: 410-706-5328; FAX: 410-706-6205. kkotloff@medicine.umaryland.edu.

Trial Registration: ClinicalTrials.gov, identifier NCT00943631 for the Sanofi-Pasteur vaccine trial; and NCT00943488 for the CSL Limited vaccine trial

Conflicts of Interest

WHC has been a paid consultant to AlphaVax, LigoCyte Pharmaceuticals, Integrated BioTherapeutics, Toyama Chemical Co, and Functional Genetics. KME has received funding from the National Institutes of Health and the CDC to evaluate the impact of influenza vaccines and study new influenza vaccines, and has received funding from PATH through the Bill & Melinda Gates Foundation (BMGF) to evaluate potential new influenza vaccines. KMK receives funding from the BMGF to evaluate maternal influenza vaccines. LAJ has received research funding from GSK, Sanofi-Pasteur, Novartis Vaccines and Pfizer. EBW has served as an advisor, consultant, and speaker for Merck vaccines, an advisor to Novartis vaccines, a speaker for Sanofi-Pasteur, and has received funding as an investigator evaluating influenza vaccines for Medimmune and Sanofi-Pasteur. KES has received grant support from the VA and Merck for zoster vaccine studies, from Wyeth for pneumococcal vaccine studies and from NIAID for influenza vaccine studies. RJD has received funding as an investigator evaluating influenza vaccines for CSL. The remaining authors have no conflicts of interest.

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vaccines in independent, but simultaneously conducted, multi-center Phase 2 trials of identical design.

Methods—Healthy adults, stratified by age (18 to 64 years and ≥ 65 years), were randomized (1:1 allocation), in a double-blind, parallel-group design, to receive two intramuscular doses (21 days apart) of vaccine containing approximately 15 µg or 30 µg of hemagglutinin (HA). Primary endpoints were safety (reactogenicity for 8 days after each vaccination and vaccine-associated serious adverse events during the 7 month study) and immunogenicity (proportion of subjects, stratified by age, achieving a serum hemagglutination inhibition [HI] antibody titer ≥ 1:40 or a 4-fold rise in titer after a single injection of either dosage).

Results—Both vaccines were well-tolerated. A single 15 µg dose induced HI titers ≥ 1:40 in 90% of younger adults (95% confidence interval [CI] 82%–95%) and 81% of elderly (95% CI 71%–88%) who received Sanofi-Pasteur vaccine (subsequently found to contain 24 µg HA in the standard potency assay), and in 80% of younger adults (95% CI 71%–88%) and 60% of elderly (95% CI 50%–70%) who received CSL vaccine. Both vaccines were significantly more immunogenic in younger compared with elderly adults by at least one endpoint measure. Increasing the dose to 30 µg raised the frequency of HI titers ≥ 1:40 in the elderly by approximately 10%. Higher dosage did not significantly enhance immunogenicity in younger adults and a second dose provided little additional benefit to either age group.

Conclusion—These trials provided evidence for policymakers that a single 15 µg dose of 2009 A/H1N1 vaccine would likely protect most U.S. adults and suggest a potential benefit of a 30 µg dose for the elderly.

Keywords

pandemic; influenza; vaccines

1. Introduction

The U.S. national influenza pandemic strategy was put to the test in April, 2009 when the novel triple-reassortant influenza A/H1N1 virus emerged and spread rapidly throughout the world.[1] To mitigate the potential for high attack rates and mortality,[2] national advisors recommended accelerated production of sufficient monovalent 2009 H1N1 vaccine for 40 million people, targeting release by mid-September, as soon as initial clinical data became available on its safety and immunogenicity.

The National Institute of Allergy and Infectious Disease (NIAID) mobilized the Vaccine and Treatment Evaluation Units (VTEUs) to evaluate the inactivated vaccines in clinical trials. The elevated rate of hospitalization and death related to the virus among individuals younger than 65 years raised concern that a multi-dose strategy similar to that used to immunize immunologically naïve children with seasonal influenza vaccine might be required for a broader age range. Alternatively, like other novel influenza A virus vaccines, a high dose might be needed.[3]

The answers to these questions would not only inform policy, but would also determine how quickly and effectively the population could be immunized given the amount of antigen anticipated. When clinical test lots of vaccine became available, we initiated two simultaneous multicenter trials in healthy adults to evaluate the safety and immunogenicity of vaccine produced by two different manufacturers and given as two doses containing either 15 or 30 µg of hemagglutinin (HA). The data from our two trials confirm and build upon a body of work already available.[4–6]

2. Methods

2.1. Study Design

Two prospective multicenter Phase 2 vaccine trials were conducted simultaneously using the identical randomized, double-blind, age-stratified, parallel-group study design, but the trials were performed independently at different clinical sites. Sanofi-Pasteur vaccine was evaluated at University of Maryland School of Medicine, Vanderbilt University School of Medicine, and Duke University School of Medicine; CSL vaccine was assessed at the University of Iowa Carver College of Medicine, Group Health Cooperative, and University of Washington School of Medicine.

In each trial, an electronic data system (EMMES Corporation, Rockville, MD, USA) assigned subjects to receive two intramuscular injections, spaced 21 days apart, of study vaccine containing either 15 µg or 30 µg of HA (1:1 allocation, in blocks of 2 and 4), stratified by trial site and age group (18 to 64 years and ≥ 65 years). Subjects and research staff who evaluated volunteers remained blinded, while designated unblinded nurses who administered the requisite dosage were not involved in any other study activity. Both study protocols were reviewed by the U.S. Food and Drug Administration (FDA) and approved by the respective Institutional Review Boards of each clinical site before enrollment began.

2.2 Study Participants

Healthy, nonpregnant adults were recruited from the community, with the goal of enrolling 200 subjects per trial into each age stratum, and written informed consent was obtained. Eligible subjects could have a chronic medical illness if stable, but not known immunosuppression, active neoplasia, egg allergy, previous severe reaction to influenza vaccines including Guillain-Barré Syndrome, or known 2009 H1N1 infection or vaccine. Receipt of seasonal influenza vaccine was acceptable if separated from test product administration by 2 weeks (inactivated) or 4 weeks (live attenuated).

2.3 Vaccines

Sanofi-Pasteur (Swiftwater, PA, USA) and CSL Biotherapies (Parkville, Australia) prepared the clinical test lots of inactivated monovalent unadjuvanted split-virus H1N1 influenza vaccines from the reference reassortant A/California/07/2009 NYMC X-179A strain provided by the Centers for Disease Control and Prevention. The viruses were propagated in embryonated chicken eggs, inactivated, and split using the same procedures that each company employs to manufacture licensed seasonal influenza vaccine. Sanofi-Pasteur vaccine contained thimerosal but CSL vaccine did not. Each company provided the two requisite concentrations per 0.5 mL (15 µg and 30 µg of HA) as measured by high-performance liquid chromatography (HPLC). When the biological reagents became available to perform single radio immunodiffusion (SRID), the standard potency assay for inactivated influenza vaccine, the assigned 15 µg and 30 µg dosages of Sanofi-Pasteur contained 24 µg and 51 µg of HA, respectively, whereas estimates for CSL vaccine by SRID and HPLC were identical.

2.4 End points

The pre-specified primary endpoints for safety were the frequency of: 1) solicited local and systemic reactogenicity for 8 days after vaccination; and 2) vaccine-associated serious adverse events (SAEs) during the 6 month study period. Co-primary immunogenicity endpoints were the proportion of subjects, stratified by age, achieving a serum HI antibody titer ≥ 1:40 or seroconversion (4-fold or greater rise in HI antibody titer) after a single injection of either 15 µg or 30 µg HA. The frequency of these responses after both inoculations comprised the secondary immunogenicity endpoints.

2.5 Study Procedures and Data Collection

For 8 days after each vaccination subjects recorded their oral temperature, as well as systemic solicited symptoms (feverishness, malaise, myalgia, headache, and nausea) and local signs (pain, tenderness, and swelling/induration) on a standardized memory aid, grading event severity using a pre-defined scale. Unsolicited AEs were documented for 6 weeks and new-onset chronic medical conditions and SAEs through 7 months. Serum samples were obtained immediately before and on days 8 (+2) and 21 (+3) after each vaccination. Data were entered into an electronic database and serum samples were shipped to the sponsor's repository within 24 hours of collection.

2.6 Laboratory Assays

Serum samples were analyzed in blinded fashion by hemagglutination inhibition (HI) assay at Southern Research Institute, Birmingham, AL, as described.[7] Each sample was tested at least twice with repeats as needed. After receptor-destroying enzyme treatment, the initial dilution in the series was defined as 1:10, following FDA recommendations. Day 0, 8 and 21 serum samples from a subset of subjects (n=100) in each trial were tested by microneutralization (MN)[8;9] to assess the sensitivity of the HI assay.

2.6 Statistical Analysis

Solicited reactions were analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none or mild versus moderate or severe) and expressed as point estimate plus 95% confidence intervals (CIs). Logistic regression was used to test for associations with vaccine dosage and dose number.

The geometric mean titer (GMT) of repeated HI assay results (at least duplicates) in an individual were computed and used as the response for all subsequent calculations. Analyses included proportion of subjects with either a pre-vaccination titer <1:10 and a post-vaccination titer 1:40 or a pre-vaccination titer 1:10 achieving a 4-fold or greater rise in HI titer from baseline [seroconversion], proportion achieving a HI titer of 1:40 [seroprotection], and GMTs.

Immune responses were examined using log transformed titers in linear and nonlinear regression models. Dosage groups were compared within each age stratum and using stratified testing procedures. Covariates examined included: gender, receipt of prior seasonal influenza vaccination, and clinical site. In selected analyses, Sidak's method was used to control p-values for multiple comparisons. Correlations between MN and HI assumed that each study day could be treated as an independent observation.

Analyses were conducted independently within each trial and there was no formal analysis conducted to compare the two trials. All analyses (including tables/figures) were performed using SAS version 9.3, (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Demographics

Between August 7 and 21, 2009, 406 and 408 subjects were enrolled and vaccinated with the first dose of Sanofi-Pasteur and CSL vaccines, respectively; >96% of subjects subsequently received a second dose (Figure 1). Ethnicity, race and gender did not vary significantly across treatment groups or by age stratum in either trial (Table 1).

3.2. Safety and tolerability

Both vaccines were generally well tolerated. Reactogenicity during 8 days post-vaccination did not differ by age strata, dosage level, or number of doses. In neither trial were there SAEs, AEs or new chronic illnesses considered to be vaccine-related, except one instance of recurrent urticaria in a recipient of Sanofi-Pasteur vaccine.

3.2.1 Sanofi-Pasteur vaccine—During the 8 days after dose 1, 33% of subjects experienced a solicited systemic reaction (26% graded as mild, 6% moderate, and <1% severe) and 50% reported a local reaction (48% mild, 2% moderate, and <1% severe). After dose 2, 26% of subjects experienced a systemic reaction (18% graded as mild, 6% moderate, and 2% severe) and 42% reported a local reaction (38% graded as mild, 3% moderate, and 1% severe). Headache and malaise were the most frequent systemic reactions and tenderness was the most frequent local reaction reported.

3.2.2 CSL vaccine—During the 8 days after dose 1, 35% of subjects experienced a solicited systemic reaction (25% graded as mild, 10% as moderate, and <1% as severe) and 50% reported a local reaction (43% graded as mild, 6% as moderate, and 1% as severe). After dose 2, 31% of subjects reported a solicited systemic reaction (24% mild, 6% moderate, and <1% severe); 46% of subjects reported a local reaction (43% mild, 3% moderate, and <1% severe). Headache and malaise were the most common systemic reactions and tenderness was the most common local reaction reported with any severity in either dosage group.

3.3. Immunogenicity

Correlation between the HI and MN assays was robust. The Spearman's correlation was 0.91 (Sanofi-Pasteur vaccine) and 0.89 (CSL vaccine).

3.3.1 Sanofi-Pasteur vaccine—Before vaccination, a higher proportion of elderly than younger adults had HI titers 1:40 (24% vs. 11%, $p=0.001$, χ^2 test). Vaccination induced robust responses in most subjects by 8 days after dose 1 (Table 3, Figure 2). Twenty-one days after the first 15 μg dose, 90% of younger adults (95% CI 82%–95%) and 81% of the elderly (95% CI 71%–88%) achieved an HI titer 1:40, while 86% of younger adults (95% CI 78%–92%) and 67% of the elderly (95% CI 57%–76%) seroconverted. The second dose conferred little additional benefit. With aggregate data at each time point post-vaccination, younger adults had significantly higher HI GMTs ($p<0.001$, t-test of log response with Sidak adjustment) and rates of seroconversion ($p=0.001$ and 0.04 were the upper bounds for the t-test and seroconversion test respectively) than the elderly (Table 2).

The impact of dosage, gender, study site, and prior seasonal influenza immunization on HI titers at each time point were explored using stratum-specific linear models (controlling for baseline HI titers). The only factor that consistently impacted younger subjects was receipt of seasonal influenza vaccine in the previous year (reported by 25% of younger subjects), which significantly reduced HI GMTs at every time-point post vaccination (day 8: $p=0.02$, day 21: $p=0.002$, and days 29 and 42: $p<0.001$). The small number of previously unvaccinated elderly (5.9%) precluded a similar analysis. The only factor consistently impacting the elderly was dosage, whereby 30 μg induced significantly higher HI GMTs 21 days after dose 1 and 8 days after dose 2 ($p=0.02$ and 0.04 with Sidak adjustment, respectively) in addition to a consistent (but statistically insignificant) trend toward a higher frequency of seroconversion and seroprotective responses (Table 3, Figure 2). Twenty-one days after receiving a single 30 μg dose, HI titers 1:40 were seen in 92% of young adults (95% CI 85%–96%) and 91% of elderly recipients (95% CI 84%–96%).

3.3.2 CSL vaccine—Most volunteers in both age strata had low pre-vaccination titers, but responded briskly to vaccination, reaching immunogenicity endpoints by day 8 (Table 4, Figure 2). Twenty-one days after a single 15 µg dose, 80% of younger adults (95% CI 71%–88%) and 60% of the elderly (95% CI 50%–70%) achieved an HI titer 1:40, while 75% (95% CI 66%–83%) and 54% (95% CI 44%–64%) of each respective stratum seroconverted. A second dose produced a minimal (2–8%) increase in the proportion with protective titers on day 42 (Table 4). At every time point post-vaccination, younger adults had higher rates of seroconversion and seroprotection (both $p < 0.001$) than the elderly (Table 4).

At every time point post-vaccination, and in both age strata, the 30 µg dosage elicited stronger immune responses by all parameters (Table 4, Figure 2). However, in stratum-specific regression models (controlling for baseline HI titers), a statistically significant benefit of the higher dosage was seen only in the elderly, and only after dose 2 ($p = 0.04$ and 0.02 for days 29 and 42, respectively). A statistically insignificant trend toward lower GMTs among prior seasonal influenza vaccine recipients was apparent (Table 2).

4. Discussion

Our experience illustrates the ability of federal, industry, and academic partners to collaborate on the rapid development, testing, and approval of vaccines for mass vaccination of the population in accordance with the U.S. national pandemic preparedness strategy. Just 4 months after the pandemic H1N1 strain was first identified, clinical trials of monovalent inactivated vaccine manufactured by two companies were initiated at six academic VTEU sites. On August 18 (11 days later), NIAID announced that an independent safety review of data from more than 500 subjects identified no safety concerns,[10] thus enabling testing to proceed in pediatric populations. On September 11, NIAID reported that a single 15 µg dose of inactivated vaccine was sufficient to induce a robust immune response in the vast majority of adults aged 18 to 64 years and in more than half of adults aged 65 and older. [11] On September 15, the FDA approved four 2009 H1N1 vaccines, including the two described herein, and on October 5, 2009, public distribution of the vaccines for the National Pandemic Vaccination Campaign was initiated.

The two trials that we conducted were not designed to be analyzed together; study sites did not overlap, HA quantities in the vaccines differed (as measured by SRID), and the randomized allocation of each vaccine was performed independently. Nonetheless, our final analysis reported herein supports the interim findings that a single dose of either inactivated monovalent vaccine appears safe and well tolerated. The genetic similarity between 2009 and 1976 H1N1 swine strains initially raised concerns that the vaccine could trigger Guillain-Barré Syndrome; however, no episodes were identified in our trial or in numerous post-licensure studies,[12–15] with the possible exception of a preliminary report from the CDC.[14]

Both vaccines induced vigorous immune responses in adults and most subjects achieved seroprotective levels within 8 days of vaccination. The brisk immune responses observed 8 days after the first vaccination are characteristic of a so-called “anamnestic response” and indicate that most adults were immunologically primed based on previous exposure to a similar antigen, despite the low pre-immunization HI titers. The current FDA guidance for licensure recommends that for children and adults < 65 years of age, the lower bound of the two-sided 95% CI for the proportion of subjects achieving HI seroconversion should be 40% and HI seroprotection should be 70%; for adults > 65 years of age, the criteria are 30% (seroconversion) and 60% (seroprotection). [16] A single dose of 15 µg of either monovalent vaccine exceeded these criteria in both age groups. Moreover, comparable immunogenicity was measured by MN, a functional assay that assesses the ability of

antibody to block viral infection in cell culture. Ultimately, our conclusion that the vaccines would likely elicit protective immunity was confirmed by post-licensure effectiveness observations. A study of four U.S. communities conducted by the U.S. Flu-VE Network demonstrated 89% effectiveness (95% CI 15%–99%) among older children and adults 10–49 years of age who were vaccinated more than 14 days prior to illness onset; 61% of these subjects received Sanofi-Pasteur vaccine and 8% received CSL vaccine.[17]

The lower antibody responses that we observed in both trials among the elderly, when compared to the younger age stratum, are consistent with published experience using monovalent, inactivated, unadjuvanted 2009 H1N1 Sanofi-Pasteur[5] and CSL vaccine.[6] Unfortunately, post-licensure surveillance among adults 50 years or older has not elucidated how this level of response might translate into vaccine efficacy during programmatic implementation since attack rates of illness in the elderly were low (presumably because of baseline immunity) and this group was not initially targeted for mass vaccination[17]. Nonetheless, we are encouraged by our observations that the lower immunogenicity in the elderly might be improved by administering a higher dose of vaccine, which was demonstrated most convincingly by the significantly higher GMTs among elderly recipients of the high dose Sanofi-Pasteur vaccine, which contained 51 µg of HA by SRID. A higher dose trivalent inactivated influenza vaccine (TIV) (Fluzone High-Dose®), containing 60 µg of HA per strain, was recently licensed exclusively for persons age ≥ 65 years, based on the elicitation of higher HI titers.[18–21] In part because of insufficient data demonstrating greater protection from influenza infection (these studies are ongoing), national advisory committees have not expressed a preference for the higher dose versus the standard dose of TIV containing 15 hemagglutinin per strain.[22]

It is notable that the immune responses reported by Plennevaux [5] and Talaat [6] appear to be stronger than those observed in our study, particularly among the elderly, despite administration of the same formulation of Sanofi-Pasteur [5] and CSL vaccine [6] as in our study. The higher post-vaccination responses are not entirely unexpected, as the proportion of subjects with seroprotective titers at baseline was several-fold higher among young adults (both trials) and elderly recipients of the CSL vaccine compared with those observed in our trial, suggesting that differences in HI assay performance characteristics may be contributory. Low inter-laboratory reliability is well-described for the HI assay, and has been attributed to differences in viral strains and red blood cell types, and the presence of non-specific inhibitors in the assay medium.[23] Additional contributing factors can include small sample sizes, which affect the precision of the estimates of immune responses, and dissimilarities among the groups under comparison with regard to age distribution (particularly when groups are defined by a broad age range) and exposure to the same or similar antigens through earlier vaccination or natural infections. Nonetheless, the observation by Talaat *et al* that post-vaccination GMTs among the elderly meet or exceed those achieved in the young adult population is unexpected. In contrast, our findings and those of others that compared the response to monovalent, inactivated, non-adjuvanted H1N1 vaccine among similar age groups of young and elderly adults found significantly lower GMTs with increasing age.[5;24]

Younger adults participating in the Sanofi-Pasteur study who received seasonal influenza vaccination in the prior year had significantly diminished immune responses and a similar trend that was not statistically significant was observed with the CSL vaccine. Our study was not designed to assess this potential relationship (the history was by subject report only, and subjects were not stratified or randomized based on vaccination history) so biased effects may be present. Nonetheless, similar immunological interference has been reported in individuals who received seasonal trivalent inactivated influenza vaccine 21 days prior to pandemic H1N1 vaccine.[25] Whereas, other studies failed to identify significant

differences [26] or observed improved responses [27;28] with co-administration of pandemic and seasonal vaccine. Mechanisms for this immunologic interference have been postulated,[25] but its occurrence and impact on protective efficacy requires further investigation.

The collective experience of our trials affords an opportunity to examine the challenges in implementing the pandemic preparedness strategy. There is a need for methods to abbreviate vaccine production times to allow more flexibility in production capacity and afford sufficient opportunity for clinical trials to be performed and clinical test lots of vaccine to be released after an emerging pandemic is identified.[29] One rate-limiting step is production of biologic agents for the SRID potency assay which under normal circumstances takes at least 6 weeks.[30;31] To meet accelerated timelines, HPLC was approved as an alternative measure; however, the HA concentration in the Sanofi-Pasteur product as measured by HPLC was later determined to be 1.6–1.7 fold higher than the concentration measured by SRID whereas the CSL product demonstrated similar HPLC and SRID results. Since the 2009 H1N1 vaccines licensed by the FDA were standardized using SRID to contain 15 µg HA per 0.5 mL dose, there may be limitations in our ability to generalize our clinical trial results to the licensed Sanofi-Pasteur vaccine. Another challenge we faced was that, in the interest of standardizing the immunological assays across trials, a single laboratory was contracted to perform the HI and MN antibody measurements for all the VTEU trials. Broadening this capacity by including several laboratories with cross-validated assays might provide necessary redundancy and ensure more expeditious availability of results. Finally, our trials generated intense media attention at local, national, and international levels. This proved to be an effective mechanism for communicating our efforts to the public, and provided an opportunity to engage the media in community education and minimize common misconceptions about influenza vaccines, vaccine safety and the public health benefits of conducting clinical trials.

In conclusion, two independent randomized, double-blind, multi-center clinical trials performed in the NIAID-sponsored VTEUs demonstrated the safety and immunogenicity of unadjuvanted, inactivated, monovalent 2009 influenza A/H1N1 vaccine after a single 15 µg dose in adults 18 years of age and older. Preliminary results generated within 6 months after the pandemic emerged were used to inform public health recommendations. The more modest responses seen among elderly subjects could potentially be improved with the use of a higher vaccine dosage.

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Highlights

- Two independent randomized, double-blind, multi-center clinical trials were performed
- Preliminary results generated within 6 months after the pandemic emerged were used to inform public health recommendations
- Both vaccines appeared safe and well-tolerated and elicited a robust HI antibody response after a single 15 µg dose
- The more modest responses seen among elderly subjects could potentially be improved with the use of a higher vaccine dosage.

Figure 1 A

Figure 1 B **Figure 1.**

Trial Profiles: A (Sanofi-Pasteur Vaccine Trial); B (CSL Biotherapies Vaccine Trial)

*Not analyzable for immunogenicity after deviation but remained in safety analysis

† Analyzable only for safety days 0–7 (no immunogenicity)

††Received dose 2, analyzable for safety on all days and for immunogenicity at all other time points

¥Remained analyzable for all analyses except day 29 immunogenicity

Figure 2 A

Figure 2 B

Figure 2 C

Figure 2 D

Figure 2. Reverse Cumulative Distribution Curves of HI antibody responses summarizing the proportion of subjects with a titer value greater than or equal to a given value for that day Curves are shown separately for the 15 μ g and 30 μ g dosages. Panel A shows Sanofi-Pasteur vaccine, 18–64 year old age stratum; B denotes Sanofi-Pasteur vaccine, $\geq$ 65 year old age stratum; C denotes CSL vaccine, 18–64 year old age stratum; and D denotes CSL vaccine, $\geq$ 65 year old age stratum.

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TABLE 1

Demographic features of participants, by age stratum and vaccine trial

	Sanofi-Pasteur Vaccine Trial			CSL Biotherapies Vaccine Trial		
	18–64 years		65 years	18–64 years		65 years
	15 µg (N= 102)	30 µg (N= 100)	15 µg (N= 100)	30 µg (N= 104)	15 µg (N= 98)	30 µg (N= 106)
Gender – N (%) Male	45 (44)	44 (44)	54 (54)	48 (46)	40 (41)	50 (47)
Ethnicity: N (%) Hispanic	2 (2)	3 (3)	1 (1)	0	2 (2)	1 (1)
Race – N (%)						
Asian	2 (2)	3 (3)	0	1 (1)	3 (3)	2 (2)
Black/African American	7 (7)	4 (4)	5 (5)	4 (4)	2 (2)	2 (2)
White	90 (88)	89 (89)	93 (93)	97 (93)	89 (91)	95 (90)
Other or unknown	3 (3)	4 (4)	2 (2)	2 (2)	4 (4)	7 (7)
Age						
Median	47.2	50.2	72.0	71.7	48.9	69.9
Min, Max	(18, 64)	(19, 64)	(65, 88)	(65, 85)	(18, 64)	(65, 91)

TABLE 2

HI GMT Responses by Dosage*, Age and Prior Receipt of Seasonal Influenza Vaccine

Study Group	Day 0		Day 8		Day 21		Day 8 Post Dose 2		Day 21 Post Dose 2	
	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)
Sanofi-Pasteur vaccine										
No seasonal influenza vaccine in previous year										
15 µg Age 18–64 yr	22	9.5 (6.0–15)	22	445.5 (238.5–831.9)	21	695.1 (466.5–1035.7)	21	706.6 (492.9–1013.1)	21	640 (439.1–932.7)
30 µg Age 18–64 yr	28	7.6 (5.5–10.5)	28	425.4 (233.3–775.1)	26	524 (288.5–951.7)	27	665.1 (454.5–973.5)	27	608 (414.3–892.1)
15 µg Age 65 yr	6	6.1 (4.4–8.4)	6	63.5 (11.2–358.7)	6	71.3 (12.2–415)	6	71.3 (13.9–366.4)	6	134.5 (35.4–512)
30 µg Age 65 yr	6	8.9 (3.8–20.9)	6	538.2 (147.2–1967.1)	6	761.1 (273.8–2115.5)	6	640 (212.4–1928.8)	6	538.2 (188.7–1534.6)
Received seasonal influenza vaccine in previous year										
15 µg Age 18–64 yr	77	8.7 (7.0–10.9)	77	160.7 (107–241.4)	77	197.7 (136.2–287)	72	207.5 (142.8–301.5)	72	174.5 (120.5–252.6)
30 µg Age 18–64 yr	72	8.9 (7.1–11.2)	72	327.8 (222.3–483.4)	72	355.7 (246–514.5)	68	308.8 (208.3–457.8)	68	274.6 (189.6–397.8)
15 µg Age 65 yr	94	13.1 (10–17.1)	94	98 (69.4–138.2)	92	125.6 (87.7–179.8)	88	141.6 (101–198.6)	89	141.3 (102.3–195)
30 µg Age 65 yr	98	13.5 (10.1–17.9)	98	136.5 (95.7–194.5)	97	214.2 (157.9–290.7)	94	225.4 (166.1–305.9)	95	196.3 (146.4–263.1)
CSL Vaccine										
No seasonal influenza vaccine in previous year										
15 µg Age 18–64 yr	26	8.5 (5.7–12.8)	26	125.9 (61.0–259.7)	26	176.4 (86.5–359.8)	26	190.3 (92.8–390.3)	26	208.9 (109.6–398.1)
30 µg Age 18–64 yr	21	6.5 (4.3–9.8)	21	237.8 (120.1–470.6)	21	201.6 (109.1–372.4)	21	262.5 (138.2–498.8)	21	196.1 (105.9–363.1)
15 µg Age 65 yr	8	7.7 (4.4–13.4)	8	70.3 (20.4–242.4)	8	47.6 (8.8–255.8)	8	103.7 (32.3–333.2)	8	83.5 (20.0–349.6)
30 µg Age 65 yr	3	7.9 (1.1–58.0)	3	160.0 (67.6–378.5)	3	160.0 (28.6–895.2)	3	127.0 (34.1–473.1)	3	127.0 (34.1–473.1)
Received seasonal influenza vaccine in previous year										
15 µg Age 18–64 yr	76	9.3 (7.5–11.6)	76	95.1 (66.5–136.2)	76	129.1 (89.8–185.8)	73	142.8 (100.7–202.3)	73	146.0 (106.1–200.7)
30 µg Age 18–64 yr	74	9.0 (6.8–11.8)	74	145.0 (99.8–210.7)	73	164.6 (113.7–238.4)	72	186.6 (129.9–268.1)	72	169.0 (117.9–242.2)
15 µg Age 65 yr	92	10.3 (8.1–13.1)	92	50.7 (36.0–71.5)	90	55.2 (38.0–80.3)	88	61.7 (42.3–90.0)	88	60.0 (42.4–84.9)
30 µg Age 65 yr	102	10.5 (8.2–13.3)	102	58.5 (41.7–82.0)	101	80.6 (57.1–113.7)	101	95.6 (68.9–132.6)	100	96.9 (70.2–133.7)

* As measured by high-performance liquid chromatography (HPLC). Corresponding measurements for the 15 µg and 30 µg doses of the Sanofi-Pasteur vaccine by the standard single radio immunodiffusion (SRID) potency assay were 24 µg and 51 µg of hemagglutinin respectively, whereas estimates for CSL vaccine by SRID and HPLC were identical.

TABLE 3
 HI Responses among Subjects Who Received Sanofi-Pasteur Vaccine on Days 0 and 21

	Day 0	Day 8	Day 21		Day 8 Post Dose 2	Day 21 Post Dose 2
Response by HI Geometric Mean Titer						
Study Group *	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)
15 µg Age 18–64 yr	101	8.9 (7.3- 10.8)	101	259.9 (189.6- 356.3)	95	271.6 (198.6- 371.2)
30 µg Age 18–64 yr	100	8.5 (7.1- 10.2)	100	352.6 (255.7- 486.3)	98	384 (282.7- 521.8)
15 µg Age 65 yr	100	12.5 (9.7- 16.1)	100	95.5 (68.5- 133.1)	94	135.5 (97.8- 187.9)
30 µg Age 65 yr	104	13.1 (10- 17.2)	104	147.7 (104.8- 208.3)	100	240 (178.8- 322.2)
Response by Proportion with Four-fold or greater rise in HI from baseline						
Study Group	N	Responders (95% CI)	N	Responders (95% CI)	N	Responders (95% CI)
15 µg Age 18–64 yr	101	0.79 (0.70- 0.87)	100	0.86 (0.78- 0.92)	95	0.88 (0.8- 0.94)
30 µg Age 18–64 yr	100	0.85 (0.76- 0.91)	98	0.89 (0.81- 0.94)	95	0.86 (0.78- 0.93)
15 µg Age 65 yr	100	0.63 (0.53- 0.72)	98	0.67 (0.57- 0.76)	94	0.72 (0.62- 0.81)
30 µg Age 65 yr	104	0.66 (0.56- 0.75)	103	0.81 (0.72- 0.88)	100	0.84 (0.75- 0.91)
Response by Proportion achieving HI 1:40						
Study Group	N	Responders (95% CI)	N	Responders (95% CI)	N	Responders (95% CI)
15 µg Age 18–64 yr	101	0.14 (0.08- 0.22)	100	0.90 (0.82- 0.95)	95	0.93 (0.85- 0.97)
30 µg Age 18–64 yr	100	0.09 (0.04- 0.16)	98	0.92 (0.85- 0.96)	95	0.92 (0.84- 0.96)
15 µg Age 65 yr	100	0.24 (0.16- 0.34)	100	0.81 (0.71- 0.88)	94	0.85 (0.76- 0.92)
30 µg Age 65 yr	104	0.24 (0.16- 0.33)	103	0.91 (0.84- 0.96)	100	0.92 (0.85- 0.96)

* Assigned dosages were measured by high-performance liquid chromatography (HPLC). Antigen content in the 15 µg and 30 µg doses of Sanofi-Pasteur vaccine, measured by the standard single radio immunodiffusion (SRID) potency assay were 24 µg and 51 µg of hemagglutinin respectively, whereas estimates for CSL vaccine by SRID and HPLC were identical.

Table 4

HI Responses among Subjects Who Received CSL Vaccine on Days 0 and 21

	Day 0	Day 8	Day 21	Day 8 Post Dose 2	Day 21 Post Dose 2
Response by HI Geometric Mean Titer					
Study Group*	N	GMT (95% CI)	N	GMT (95% CI)	N
15 µg Age 18–64 yr	102	9.1 (7.6–11.0)	102	139.8 (101.4–192.7)	99
30 µg Age 18–64 yr	97	8.7 (6.8–11.0)	96	179.6 (131.4–245.6)	94
15 µg Age 65 yr	102	9.9 (8.0–12.3)	100	55.5 (38.9–79.3)	97
30 µg Age 65 yr	105	10.4 (8.2–13.2)	104	82.2 (58.7–115.0)	103
Response by Proportion with Four-fold or greater rise in HI from baseline					
Study Group	N	Responders (95% CI)	N	Responders (95% CI)	N
15 µg Age 18–64 yr	102	0.70 (0.60–0.78)	102	0.75 (0.66–0.83)	99
30 µg Age 18–64 yr	97	0.77 (0.68–0.85)	96	0.81 (0.72–0.88)	94
15 µg Age 65 yr	102	0.49 (0.39–0.59)	100	0.54 (0.44–0.64)	97
30 µg Age 65 yr	105	0.55 (0.45–0.65)	104	0.60 (0.50–0.69)	103
Response by Proportion achieving HI 1:40					
Study Group	N	Responders (95% CI)	N	Responders (95% CI)	N
15 µg Age 18–64 yr	102	0.13 (0.07–0.21)	102	0.80 (0.71–0.88)	99
30 µg Age 18–64 yr	97	0.10 (0.05–0.18)	96	0.86 (0.78–0.93)	94
15 µg Age 65 yr	102	0.16 (0.09–0.24)	100	0.60 (0.5–0.7)	97
30 µg Age 65 yr	105	0.17 (0.1–0.26)	104	0.71 (0.61–0.8)	103

* Assigned dosages were measured by high-performance liquid chromatography (HPLC). Antigen content in the 15 µg and 30 µg doses of Sanofi-Pasteur vaccine, measured by the standard single radio immunodiffusion (SRID) potency assay were 24 µg and 51 µg of hemagglutinin respectively, whereas estimates for CSL vaccine by SRID and HPLC were identical.