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Phase II Trial in Adults of Concurrent or Sequential 2009 Pandemic H1N1 and 2009-2010 Seasonal Trivalent Influenza Vaccinations

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Abstract

Background—During the 2009 influenza pandemic both seasonal and 2009 pandemic vaccines were recommended. We conducted a randomized trial of monovalent 2009-H1N1 vaccine and seasonal trivalent inactivated influenza vaccine (IIV3) given sequentially or concurrently to adults.

Methods—Adults randomized to 4 study groups and stratified by age (18-64 and ≥ 65 years) received 1 dose of seasonal IIV3 or placebo and 2 doses of 2009-H1N1 vaccine or placebo in one of 4 combinations, i.e., H1N1+Placebo/H1N1+Placebo/**IIV3** (HP/HP/**V3**), H1N1+ **IIV3**/H1N1+Placebo/Placebo (**HV3**/HP/P), H1N1+Placebo/H1N1+ **IIV3**/Placebo (HP/**HV3**/P), and **IIV3**+Placebo/H1N1+Placebo/H1N1 (**V3P**/HP/H). Intramuscular injections were given three times

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at 21 day intervals. Sera for antibody assays were obtained prior to and 21 days after each vaccination. Reactogenicity and adverse events were monitored.

Results—Eight hundred-five (805) adults were enrolled. All combinations of vaccines were safe and well tolerated. In general, one dose of 2009-H1N1 and one dose of IIV3, regardless of sequence or concurrency of administration, were immunogenic in adults. There were no significant differences in geometric mean titers (GMT) or the proportions of subjects with 4-fold rise in antibody responses and titers 40 for any vaccine group or between age strata for 2009-H1N1 after the first or second dose, although the vaccine sequence affected the titers to the IIV3 antigens. Hemagglutination inhibition antibody (HAI) GMTs against 2009-H1N1 for the combined age strata 21 days after the first 2009-H1N1 dose were 190.4, 182.1, 232.9 and 157.5 for HP/HP/V3, HV3/HP/P, HP/HV3/P and V3P/HP/H, respectively. While IIV3 GMTs were adequate they were generally lower than the 2009-H1N1 GMTs. In a subset of subjects, there was good correlation between HAI and microneutralization (MN) titers (Spearman's correlation coefficient 0.92).

Conclusions—All vaccine combinations were generally well tolerated. Immune responses to one dose of 2009-H1N1 were adequate regardless of the sequence of vaccination in all age groups, but the sequence affected titers to IIV3 antigens.

Keywords

Influenza vaccine; 2009-H1N1; seasonal IIV3; pandemic; adults; elderly; concurrent; sequential; HAI; microneutralization

Introduction

During April 2009, the pandemic 2009-H1N1 influenza virus (A/California/7/09) was identified as a novel influenza strain(1-4). Although children and young adults had little pre-existing antibody to this virus, some studies found older adults did have pre-existing antibody to 2009-H1N1(5-7). Concern about the potential impact of the 2009-H1N1 virus led to rapid evaluation of a monovalent pandemic H1N1 vaccine in adults and children(8-17). This study was designed to inform U.S. policy by determining whether the receipt of pandemic monovalent 2009-H1N1 inactivated influenza vaccine (2009-H1N1) concurrently with, prior to, or following licensed seasonal inactivated influenza vaccine (IIV3) affected the reactogenicity or antibody responses for either vaccine in adults aged 18 years.

Methods

Vaccines

The split-virion 2009 pandemic influenza vaccine (Sanofi Pasteur, one lot, UD12415) contained 15 µg/0.5mL of H1 hemagglutinin (HA) [A/California/7/09 (H1N1)-like virus] based on high performance liquid chromatography (HPLC) potency testing. Subsequent testing with single radial immunodiffusion (SRID) found the potency of the vaccine to be 22-25µg/0.5mL. The 2009-2010 IIV3 (Sanofi Pasteur, one lot, U3189AA) contained 15µg HA each of A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 [A/Brisbane/10/2007

(H3N2)-like virus] and B/Brisbane/60/2008. The placebo was normal saline. All injections were administered as a single 0.5 mL intramuscular injection into the deltoid muscle; one per arm.

Subjects and Study design

Subjects, 18 years of age, were enrolled in an NIH-sponsored, randomized, placebo-controlled phase II vaccine trial conducted at 4 sites in the United States. The study was approved by the Institutional Review Board of each of the participating sites and all subjects provided informed consent. Subjects were randomized in a 1:1:1:1 ratio to 4 groups (Figure 1), stratified by age [planned 200 subjects per group with 100 subjects per age-stratum (18-64 or 65 years)], to receive 1 dose of IIV3 or placebo and 2 doses of 2009-H1N1 vaccine or placebo in one of 4 combinations such that each subject received 2 injections (one per arm) on Days 0 and 21 and 1 injection on Day 42. The groups are as follows: H1N1+Placebo/H1N1+Placebo/IIV3 (HP/HP/V3), H1N1+ IIV3/H1N1+Placebo/Placebo (HV3/HP/P), H1N1+Placebo/H1N1+ IIV3/Placebo (HP/HV3/P), and IIV3+Placebo/H1N1+Placebo/H1N1 (V3P/HP/H).

Safety and Immunogenicity

Safety was measured by assessment of reactogenicity for 8 days and adverse events (AEs) for 21 days after each vaccination, and serious adverse events (SAEs) and new-onset chronic medical conditions for 8 months after first vaccination. HAI titers were measured prior to each vaccination and 21 days following the last vaccination. Microneutralization (MN) titers were measured against 2009-H1N1 on a subset of subjects per group prior to and 21 days after the first vaccination.

Antibody Assays

Southern Research Institute (SR) performed HAI assays(18) and MN assays(19)for 2009-H1N1 and Cincinnati Children's Hospital Medical Center performed a standard HAI assay for seasonal influenza as previously described(18). Virus reference strains were provided by the Center for Disease Control and Prevention (CDC).

Enrollment Criteria

Healthy, nonpregnant adults 18 years of age were eligible to participate if they had not previously received a 2009-2010 seasonal influenza vaccine. Women of childbearing potential used adequate contraception from the time of enrollment through 30 days after the last vaccination. Exclusions included egg allergy; acute illness; oral temperature 101°F (38.3°C) within 3 days prior to vaccination; history of Guillain-Barré Syndrome; immunosuppression, or planned travel outside of North America within the 63 days after first vaccination (full enrollment criteria available at clinicaltrials.gov NCT00943878).

Statistical Analyses

The sample size for this phase II study was powered to detect differences in immune response rates without correction for multiple comparisons. It was projected to have 80% power for a two-tail test with Type I Error=5.0% to detect different frequencies between two

vaccine groups within the same age-stratum when the true differences were more than 27%. The sample size was determined to have >95% power to observe one or more SAEs related to vaccination when the true underlying rate was 0.50%. Within a single age-stratum vaccine schedule-specific group, the power was 78% to observe one or more such events when the true rate was 3.0%.

Safety

Injection site and systemic reactogenicity summary rates were analyzed by dichotomizing the most severe response for each event over the 8 day post vaccination period into binary variables (none versus mild, moderate or severe) and using exact confidence intervals. Unsolicited AEs were reported using rates and exact 95% confidence intervals.

Immunogenicity

The proportions of subjects achieving 4-fold rise in HAI titer, an HAI titer of 40 and the geometric mean titers (GMT) at 21 days after each vaccination were evaluated. Analyses of GMTs were computed on a logarithmic scale and converted back to the original scale.

Unless otherwise noted, analyses were primarily conducted using analysis of variance for continuous variables, with results occasionally confirmed, but not reported, using standard non-parametric methods. Binary results were analyzed using logistic regression and contingency table methods. Ten subjects with unknown prior influenza vaccination status were not included in multivariate modeling.

Results

Subject Characteristics and Safety Results

Eight hundred-five (805) subjects were vaccinated between August 7 and 28, 2009. Fifty-five percent of the subjects were female, 91% were white, and 96% were non-Hispanic (Table 1). There were 408 (50.7%) subjects in the 18-64 year old group, median age 48.3 (range 18-64) years and 397 (49.3%) subjects in the 65 years old group, median age 70.4 (range 65-92) years. Overall the median age was 64.5 (range 18-92) years. Ethnicity, race, percentage of males and age did not vary significantly across treatment groups.

Forty subjects received only the first dose(s) of vaccine(s) at Day 0, and 19 subjects did not receive the third dose (Figure 1). Reasons for not receiving the full vaccine schedule included reaction to prior dose(s) (n=2); unrelated illness (n=25); no longer eligible, mostly due to new or changed medications (n=21); subject noncompliance with the visit schedule (n=10); and subject refusal (n=1). Fourteen and 11 subjects did not complete the Day 42 and/or Day 63 blood draw, respectively.

Injection Site Reactogenicity

The percentages of subjects in each treatment group who experienced any injection site reaction (pain, tenderness or swelling) in the eight days after vaccinations ranged from 71.3 - 78.0% (data not shown). Tenderness and pain were the most common injection site reactions across all groups and vaccinations.

No subjects experienced severe injection site reactions after any vaccination. Moderate injection site reactions occurred in 47 (6%) subjects after the first two injections on Day 0, 36 (4%) subjects after the second two injections on Day 21, and 22 (3%) subjects after the third injection on Day 42. There were no significant differences by vaccine group or whether vaccines were given sequentially or concurrently (Table 2 for moderate combined with severe reactogenicity and adverse event grading).

Nine (1%) subjects experienced large (>50mm) injection site erythema and/or swelling post vaccination, across all treatment groups. Only in the HV3/HP/P group did subjects report large reactions after receipt of concurrently administered vaccines, with 2 subjects reporting large swelling after receiving both products at Day 0, one in the arm that received 2009-H1N1 and one in the arm that received V3(data not shown).

Systemic Reactions

Among all subjects, 7 (0.9%) experienced severe solicited systemic reactions after vaccination, i.e., 2, 3, 1 and 1 subjects in the HP/HP/V3, HV3/HP/P, HP/HV3/P, V3P/HP/H groups, respectively (Table 2 for moderate combined with severe reactogenicity and adverse event grading). Six subjects were in the younger age stratum. No subjects reported severe fever (>39°C) after any dose. Overall, there were no significant differences within any age strata for the solicited systemic symptoms after any vaccination. In general, more subjects 18-64 years of age (47.1%-63.4%) reported solicited systemic symptoms compared to the 65 years old group (29.6%-42.6%), with more than twice the number of subjects in the younger age group reporting symptoms after the first dose compared to subjects 65 years. Across all vaccinations and study groups, headache was the most prominent systemic symptom reported followed by malaise. In contrast, subjects in the 65 years age group reported a higher frequency of malaise than headache compared to the younger age group.

Unsolicited Adverse Events

A total of 858 unsolicited nonserious AEs were reported by 465 (57.8%) subjects; of these 111 (13.8%) subjects reported events considered to be associated to vaccine. There were no significant differences in the proportion of subjects in the 4 groups experiencing events considered to be associated with vaccination between study groups. Significantly more subjects experienced AEs associated with vaccination in the younger group [78(19.1%)] than in the older group [33(8.3%)] ($p<0.001$). There were 48 severe unsolicited AEs reported over the course of the study; 36 of these events were considered to be SAEs, unrelated to study vaccines. Only two severe events, vomiting and possible tendonitis with fasciitis (both non-serious) were considered associated with vaccine. One death occurred due to acute subarachnoid hemorrhage in an 82 year-old subject.

Immunogenicity Results

2009-H1N1

Response to 2009-H1N1 after First Dose (Primary Endpoints): The proportions of subjects with 4-fold HAI antibody titer increases against 2009-H1N1 virus 21 days following the first dose of 2009-H1N1 were not significantly different when compared by

vaccination schedule. Nor was there a significant difference in response by age-strata: 85% and 75% of subjects in age strata 18-64 and ≥ 65 years, respectively, achieved a 4-fold rise response (Table 3). In the younger and older age strata, respectively, the 4-fold rise response rates for vaccine groups were HP/HP/V3 (86% and 77%), HV3/HP/P (82% and 74%), HP/HV3/P (90% and 77%), V3P/HP/H (83% and 72%). Since baseline titers were uniformly low across all groups and age strata (Table 4.a, Figure 2), the proportions of subjects achieving a serum HAI antibody titer of ≥ 40 against the 2009-H1N1 virus 21 days following the first dose of 2009-H1N1 vaccine followed the same pattern, and were just slightly higher overall, e.g., 89% and 82% percent in age groups 18-64 and ≥ 65 years, respectively (Table 3). The requirements for FDA licensure were met for 2009-H1N1 by all groups in both strata (20).

Responses to 2009-H1N1 after the Second Dose (Secondary Endpoints): There was a minimal increase in titers after the second dose of 2009-H1N1 (Table 4.a, Figure 2). The proportions of subjects with 4-fold HAI antibody titer increases against 2009-H1N1 virus 21 days following the second dose of 2009-H1N1 were not significantly different when compared across vaccination schedules. Nor was there a significant difference in response by age-strata: 88% and 78% of subjects in age groups 18-64 and ≥ 65 years, respectively, achieved a four-fold response (Table 3). In the younger stratum, the 4-fold response rates for vaccine groups HP/HP/V3, HV3/HP/P, HP/HV3/P, V3P/HP/H were 90%, 84%, 94% and 83%, respectively. In the older stratum, the 4-fold response rates for the groups were 83%, 79%, 77% and 71%, as above. Proportions ≥ 40 were again just slightly higher than proportions with 4-fold rise.

2009-H1N1 HAI GMT Responses: Prevaccination GMT against 2009-H1N1 were <10 for all 4 vaccine groups in the younger age stratum regardless of prior receipt of the 2008-2009 seasonal vaccine (Table 4.a). Prevaccination GMTs for groups receiving HP/HP/V3, HV3/HP/P, V3P/HP/H were also <10 in the older age stratum (Figure 2). However, in the HP/HV3/P group, the overall prevaccination GMT was 13.0; 13.5 and 6.2 for those with (n=94) and without (n=5) prior receipt of 2008-2009 seasonal vaccine, respectively.

GMTs 21 days after the first dose of 2009-H1N1 vaccine were greater in the younger stratum overall ($p < 0.0001$) and within each vaccination group (Table 4.a, Figure 2). GMTs by group (p-values for group-specific age stratum differences) were HP/HP/V3 (289.8 vs 126.7, $p = 0.0008$), HV3/HP/P (231.8 vs 141.7 $p = 0.07$), HP/HV3/P (285.8 vs 190.6 $p = 0.02$), and V3P/HP/H (232.5 vs 107.1 $p = 0.0007$) in subjects in the younger versus older age groups, respectively.

Similarly, GMTs 21 days after the second dose of 2009-H1N1 vaccine, were greater in the younger stratum overall ($p < 0.0001$) and within each vaccination group (Table 4.a, Figure 2). GMTs by group (p-values for group-specific age stratum differences) were HP/HP/V3 (277.1 vs 140.0, $p < 0.0001$), HV3/HP/P (199.1 vs 135.4, $p = 0.03$), HP/HV3/P (311.5 vs 194.4, $p = 0.04$), and V3P/HP/H (229.8 vs 108.1, $p = 0.0008$) in subjects in the younger versus older age groups, respectively. Results for the group initially co-administered H1N1+V3 on Day 0 were consistently lower than those receiving 2009-H1N1 alone in both age strata, but

the differences were not significant. Overall, the second dose of 2009-H1N1 did not effectively boost titers.

IIV3

Seasonal H1N1 HAI Responses (Secondary Endpoints): For both age strata, the best responses to seasonal H1N1 at 21 days post receipt of IIV3 were noted in the group receiving pandemic 2009 H1N1 alone at the first and second vaccination (Table 4.b). In the younger age stratum, the only significant differences in HAI titers for the seasonal H1N1 antigen were for four-fold rise responses (Not shown). Group HP/HP/V3 had a higher response rate than the HV3/HP/P group (49% vs 31%, $p=0.02$) or the V3P/HP/H group (49% vs 26%, $p=0.004$). In the older stratum, the four-fold response rates for the groups HP/HP/V3, HV3/HP/P, HP/HV3/P and V3/HP/H were 72%, 16%, 42% and 18%, respectively, 21 days after receipt of IIV3. The HP/HP/V3 group was statistically superior to all other groups ($p=0.004$) and the HP/HV3/P group was superior to groups HV3/HP/P and V3/HP/H ($p=0.001$). Overall, GMTs against IIV3 H1N1 strain continued to increase after the first vaccination through day 63 regardless of the timing of the IIV3 (Table 4.b).

The proportion of subjects with a titer of ≥ 40 against seasonal H1N1 21 days after vaccination with IIV3 in the younger age stratum is not statistically different between groups. The proportion of subjects with a titer of ≥ 40 in groups HP/HP/V3, HV3/HP/P, HP/HV3/P, V3P/HP/H in the older age stratum were 92%, 74%, 92% and 78%, respectively. The HP/HP/V3 and HP/HV3/P groups are each superior to groups HV3/H/P and V3P/HP/H ($p=0.001-0.01$).

GMTs against seasonal H1N1 21 days after vaccination with seasonal V3 for the younger age stratum for groups HP/HP/V3, HV3/HP/P, HP/HV3/P, V3P/HP/H were not statistically different, 88, 93, 101 and 85, respectively (Table 4.b). GMTs for the older age stratum follow a similar pattern: 140, 52, 86, and 60 (peak titers were 152, 126 and 166 at day 63), respectively. The HP/HP/V3 group was statistically superior to all other groups ($p=0.0001$) and group HP/HV3P/P was superior to groups V3/HP/P and V3P/HP/H ($p=0.01$).

H3N2: There were no significant group differences for the H3N2 antigen in either age stratum for the proportion of subjects with a 4-fold rise in titer, titers of ≥ 40 , and GMTs (Table 3, Table 4.c).

B: The only significant difference for the B antigen was the HAI GMT 21 days post V3 vaccination within the younger stratum (Table 4.d). GMTs for the V3P/HP/H group were greater than the groups receiving HP/HP/V3 (GMT=133 vs 79, $p=0.0002$) and HP/HV3/P (GMT=133 vs 103, $p=0.004$). The GMT for HV3/HP/P was 123. There were no significant differences for the B antigen in the older stratum for any parameter.

Analysis of covariance for IIV3 strain-specific log (HAI) responses modeled against baseline titer, vaccine group, clinical site, age strata and prior receipt of IIV3 did not result in significant associations for prevaccination titers, vaccination treatment group, and prior year's vaccination status for any of the 3 viral strains represented in the 2009-2010 seasonal influenza vaccine

Microneutralization—Overall, MN results against 2009-H1N1 among the subset tested at Day 0 and 21 only after first vaccination were well correlated with HAI results (data not shown). The Spearman's correlation between the 2 assays is 0.90 restricting to pairs for which at least one assay showed some detectable titer. GMTs to vaccination were high for subjects in the younger stratum receiving H1N1 vaccine in Groups HP/HP/V3, HV3/HP/P and HP/HV3/P, ranging from 370 to 453 at Day 21. They were somewhat reduced in the older stratum, ranging from 173 to 297.

Discussion

The 2009-2010 influenza pandemic provided an opportunity to evaluate the effect of the timing of administration of two recommended influenza vaccinations in adults on safety and immunogenicity. The data from this study provided information to guide U.S. policy for the co-administration of these vaccines during the 2009-2010 influenza season, and provides valuable information and insight for the development of recommendations for future influenza pandemic seasons.

The vaccines were well-tolerated. The majority of injection site and systemic reactions were mild to moderate. Less than 1% experienced severe systemic reactions related to vaccine. The favorable safety profile following the administration of 2009-H1N1 vaccine in adults is consistent with the findings in multiple trials of adjuvanted and unadjuvanted 2009-H1N1 tested as a single vaccine(9,11,12,21,22,23-31) or concurrently or sequentially administered with V3(27-31) and during surveillance during a mass vaccination campaign.

Pre-vaccination GMTs against 2009-H1N1 were low; however, adequate titers (≥ 40 in HAI assay) were achieved after one dose of vaccine in a high proportion of subjects, consistent with prior priming against H1N1 viruses, and a second dose of 2009-H1N1 did not boost GMTs. There were no significant differences in HAI titers by vaccine group or age strata for GMT, proportions with four-fold rise or titers ≥ 40 after either H1N1 vaccination. Correlation of HAI with the MN assay was high.

Of interest, the proportions of responders to seasonal H1N1 were highest when IIV3 was given as the third vaccination. A similar response was observed in other studies(31). Overall, when both age strata are combined, GMTs against IIV3 H1 continued to increase after the first vaccination through day 63 regardless of the timing of the IIV3 vaccination. The reason for this is not clear but cross clade and hetero-subtypic antibody responses may play a role. Also, a slower antibody response to IIV3 has been described in the elderly(32). In contrast, the responses to the B antigen were higher if the IIV3 was given as the first vaccine. This may be partially due to antigenic competition when 2009-H1N1 is administered first.

When multivariate analysis was performed with adjustments for vaccine group, gender, clinical site and receipt of the prior year's seasonal influenza vaccine, receipt of the prior year's seasonal influenza vaccine had little effect on 2009-H1N1 antibody titers.

Conclusions

The study was undertaken to assist in setting policy for the co-administration of the pandemic and seasonal influenza vaccines in adults. Co-administration is supported by the production of adequate HAI titers against 2009-H1N1 H1 and IIV3 antigens. Only one dose of 2009-H1N1 vaccine given concurrently with IIV3 was required to achieve levels of antibody that are associated with protection against influenza.

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Figure 1. DMID 09-0039

Figure 1 provides the study groups, and the number of subjects randomized and included in the immunogenicity analysis.

V3 = trivalent vaccine, H = 2009 H1N1, P=placebo

H1N1+Placebo/H1N1+Placebo/IIV3 = HP/HP/V3; H1N1+IIV3/H1N1+Placebo/Placebo = HV3/HP/P; H1N1+Placebo/H1N1+IIV3/Placebo = HP/HV3/P; and IIV3+Placebo/H1N1+Placebo/H1N1 = V3P/HP/H

Figure 2. Immunogenicity

Geometric mean titers 21 days after each vaccination by vaccination group and age strata for each of the 4 vaccine strains, 2009H1N1 [A/California/7/09 (H1N1) v-like virus] and IIV3 {A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 [A/Brisbane/10/2007 (H3N2)-like virus] and B/Brisbane/60/2008 virus}.

Table 1

Demographics by Demographic Data by Study Group and Age Stratum

	All Groups (N= 805)			HP/HP/V3 (N= 202)			HV3/HP/P (N= 200)			HP/HV3/P (N= 203)			V3/HP/H (N= 200)		
	All	Age 18-64 yr (N=408)	Age 65 yr (N=397)	All	Age 18-64 yr (N=101)	Age 65 yr (N=101)	All	Age 18-64 yr (N=103)	Age 65 yr (N=97)	All	Age 18-64 yr (N=102)	Age 65 yr (N=101)	All	Age 18-64 yr (N=102)	Age 65 yr (N=98)
Gender -- N(%)															
Male	361 (45)	167 (41)	194 (49)	103 (51)	44 (44)	59 (58)	82 (41)	39 (38)	43 (44)	99 (49)	44 (43)	55 (54)	77 (39)	40 (39)	37 (38)
Female	444 (55)	241 (59)	203 (51)	99 (49)	57 (56)	42 (42)	118 (59)	64 (62)	54 (56)	104 (51)	58 (57)	46 (46)	123 (62)	62 (61)	61 (62)
Ethnicity -- N(%)															
Non-Hispanic	773 (96)	380 (93)	393 (99)	194 (96)	95 (94)	99 (98)	191 (96)	95 (92)	96 (99)	194 (96)	93 (91)	101 (100)	194 (97)	97 (95)	97 (99)
Hispanic	32 (4)	28 (7)	4 (1)	8 (4)	6 (6)	2 (2)	9 (5)	8 (8)	1 (1)	9 (4)	9 (9)	0	6 (3)	5 (5)	1 (1)
Race -- N(%)															
Asian	12 (1)	12 (3)	0	2 (1)	2 (2)	0	3 (2)	3 (3)	0	3 (1)	3 (3)	0	4 (2)	4 (4)	0
Hawaiian/Pacific Islander	1 (0)	1 (0)	0	0	0	0	0	0	0	1 (0)	1 (1)	0	0	0	0
Black/African American	44 (5)	33 (8)	11 (3)	7 (3)	6 (6)	1 (1)	11 (6)	5 (5)	6 (6)	11 (5)	10 (10)	1 (1)	15 (8)	12 (12)	3 (3)
White	734 (91)	351 (86)	383 (96)	189 (94)	90 (89)	99 (98)	181 (91)	91 (88)	90 (93)	186 (92)	87 (85)	99 (98)	178 (89)	83 (81)	95 (97)
Multi-Racial	8 (1)	7 (2)	1 (0)	1 (0)	1 (1)	0	3 (2)	2 (2)	1 (1)	1 (0)	1 (1)	0	3 (2)	3 (3)	0
Other/Unknown	6 (1)	4 (1)	2 (1)	3 (1)	2 (2)	1 (1)	2 (1)	2 (2)	0	1 (0)	0	1 (1)	0	0	0
Age															
Mean (STD)	58.8 (16.2)	45.9 (12.2)	72.1 (5.6)	59.2 (16.2)	46.5 (12.6)	72.0 (6.1)	58.4 (16.7)	45.2 (12.3)	72.4 (5.6)	58.5 (16.6)	44.9 (12.3)	72.1 (5.3)	59.1 (15.3)	47.0 (11.5)	71.6 (5.5)
Median	64.5	48.3	70.4	65.0	48.4	69.9	64.1	46.6	71.1	64.9	48.6	70.5	62.8	48.7	70.1
Min,Max	(18, 92)	(18, 64)	(65, 92)	(18, 92)	(18, 64)	(65, 92)	(21, 87)	(21, 64)	(65, 87)	(20, 89)	(20, 64)	(65, 89)	(18, 88)	(18, 64)	(65, 88)

V3 = trivalent vaccine, H = 2009 H1N1, P=placebo

H1N1+Placebo/H1N1+Placebo/IIV3 = HP/HP/V3; H1N1+IIV3/H1N1+Placebo/Placebo = HV3/HP/P; H1N1+Placebo/H1N1+IIV3+Placebo = HP/HV3/P; and IIV3+Placebo/H1N1+Placebo/H1N1= V3P/HP/H

Table 2

Proportion¹ of Subjects Reporting Moderate and/or Severe Solicited Systemic and Local Reactions² in the 8 Days After Each Vaccination

Study Group (Dose 1/Dose 2/Dose 3)	Age Stratum	Vaccine 1 (%)				Vaccine 2 (%)				Vaccine 3 (%)		
		N	Systemic	Injection Site		N	Systemic	Injection Site		N	Systemic	Inj. Site IIV3 Arm
				H1N1 Arm	Placebo Arm			H1N1 Arm	Placebo Arm			
HP/HP/V ³	18-64 yr	101	15	5	4	95	9	7	1	95	5	9
	65 yr	101	6	0	0	98 ⁴	5	0	0	95	1	5
HV3/HP/P	18-64 yr	N	Systemic	Injection Site		N	Systemic	Injection Site		N	Systemic	Inj. Site Placebo Arm
				H1N1 Arm	IIV3 Arm			H1N1 Arm	Placebo Arm			
	18-64 yr	103	19	4	12	97	11	3	0	95	5	2
	65 yr	97	1	1	6	93	2	1	0	90	4	0
HP/HV3/P	18-64 yr	N	Systemic	Injection Site		N	Systemic	Injection Site		N	Systemic	Inj. Site Placebo Arm
				H1N1 Arm	Placebo Arm			H1N1 Arm	IIV3 Arm			
	18-64 yr	102	5	3	2	95	12	4	7	90	8	1
	65 yr	101	3	1	0	94	6	2	6	93	7	1
V3P/HP/H	18-64 yr	N	Systemic	Injection Site		N	Systemic	Injection Site		N	Systemic	Inj. Site H1N1 Arm
				Placebo Arm	IIV3 Arm			H1N1 Arm	Placebo Arm			
	18-64 yr	102	14	3	11	97	8	4	3	96	5	6
	65 yr	98	6	2	3	95	4	1	1	92	0	0
Vaccination visits where both H1N1 and IIV3 are given are shaded												

¹ Since N is near 100 for each group/age stratum, the number of subjects reporting moderate and/or severe events is equivalent to the percentage; therefore, only the percentage is presented.

² Reactions were graded as mild, moderate or severe. Mild events did not interfere with daily activity. Moderate events resulted in some interference with daily activity. Severe events resulted in significant interference of daily activity. Fever was graded as mild (38.0-38.4°C), moderate (38.5-38.9°C) or severe (>39°C).

³ H1N1+Placebo/H1N1+Placebo/IIV3 = HP/HP/V3; H1N1+IIV3/H1N1+Placebo/Placebo = HV3/HP/P; H1N1+Placebo/H1N1+IIV3/Placebo = HP/HV3/P; and IIV3+Placebo/H1N1+Placebo/H1N1=V3P/HP/H

⁴ Reactogenicity data for one subject was not available.

V3 = trivalent vaccine, H = 2009 H1N1, P=placebo

Table 3
HAI Antibody Titer, and Proportion of Subjects Stratified by Age^I with 1:40 Titer to
Indicated Antigen and Proportion of Subjects with 4-Fold Rise 21 Days After Receipt of
Antigen-Specific Vaccine

			Titer of 1:40 or Greater	4-Fold or Greater Increase from Baseline
	Antigen	Day Post Vaccination	Proportion Responding (95% CI)	Proportion Responding (95% CI)
Stratum	V3 B strain	21 Days Post IIV3	0.91 (0.88, 0.94)	0.46 (0.41, 0.52)
	V3 H1 strain	21 Days Post IIV3	0.87 (0.83, 0.90)	0.38 (0.33, 0.43)
	V3 H3 strain	21 Days Post IIV3	0.92 (0.89, 0.94)	0.57 (0.52, 0.62)
18-64 years	2009 Novel	21 Days Post Vac. 1	0.89 (0.86, 0.92)	0.85 (0.81, 0.89)
	H1N1	21 Days Post Vac. 2	0.92 (0.89, 0.95)	0.88 (0.84, 0.91)
	V3 B strain	21 Days Post IIV3	0.78 (0.74, 0.82)	0.18 (0.14, 0.22)
	V3 H1 strain	21 Days Post IIV3	0.84 (0.80, 0.88)	0.36 (0.32, 0.41)
65 years and older	V3 H3 strain	21 Days Post IIV3	0.93 (0.90, 0.95)	0.45 (0.40, 0.50)
	2009 Novel H1N1	21 Days Post Vac. 1	0.82 (0.78, 0.86)	0.75 (0.71, 0.79)
		21 Days Post Vac. 2	0.84 (0.80, 0.88)	0.78 (0.73, 0.82)

V3 = trivalent vaccine, H = 2009 H1N1, P=placebo

^I All 4 vaccine groups are combined

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Table 4.b
Geometric Mean Titers (95% Confidence Intervals) as Assessed by the Hemagglutination Inhibition Assay, IIV3 H1 Antigen

All Subjects*		Day 0		Day 21		Day 42		Day 63	
Study Group	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N
HP/HP/V3	199	31.0 (27.2- 35.4)	199	37.2 (32.7- 42.3)	189	71.7 (62.7- 81.9)	183	110.8 (95.7- 128.3)	
HV3/HP/P	198	34.5 (30.1- 39.7)	198	70.3 (60.0- 82.3)	184	105.3 (91.4- 121.4)	176	121.4 (103.9- 141.9)	
HP/HV3/P	200	34.3 (30.6- 38.5)	200	41.7 (37.2- 46.7)	183	92.7 (80.9- 106.4)	178	104.3 (90.3- 120.4)	
V3P/HP/HP	196	36.2 (32.0- 41.0)	196	71.7 (62.2- 82.6)	188	105.9 (93.2- 120.2)	181	124.7 (108.1- 144.0)	
Subjects Age 18 – 64 Years									
HP/HP/V3	98	33.0 (26.5- 41.1)	98	39.2 (31.9- 48.1)	94	68.5 (56.1- 83.7)	91	87.7 (71.1- 108.0)	
HV3/HP/P	101	36.8 (29.9- 45.4)	101	93.0 (73.2- 118.3)	92	121.1 (98.1- 149.5)	89	97.2 (77.6- 121.8)	
HP/HV3/P	99	33.6 (28.4- 39.7)	99	41.1 (35.1- 48.2)	90	100.8 (81.3- 125.0)	88	85.9 (71.0- 103.9)	
V3+P/HP/HP	99	35.8 (29.8- 42.9)	99	85.2 (69.0- 105.2)	94	114.8 (94.8- 139.1)	90	93.3 (76.8- 113.3)	
Subjects Age 65 Years and Older									
HP/HP/T V3	101	29.2 (25.0- 34.0)	101	35.4 (30.2- 41.4)	95	74.9 (62.6- 89.7)	92	139.7 (114.7- 170.2)	
HV3/HP/P	97	32.3 (26.9- 38.8)	97	52.5 (43.3- 63.6)	92	91.6 (75.7- 110.9)	87	152.5 (123.8- 187.9)	
HP/HV3/P	101	35.1 (29.9- 41.2)	101	42.3 (35.8- 49.8)	93	85.6 (71.9- 101.8)	90	126.0 (101.9- 155.8)	
V3P/HP/HP	97	36.7 (31.0- 43.5)	97	60.1 (50.0- 72.3)	94	97.6 (82.5- 115.6)	91	166.2 (136.6- 202.3)	

* Slight differences arise between the tables containing all per protocol subjects and the numbers quoted in the text that are a result of statistical modeling that drops 10 subjects who did not know if they had received the prior year's seasonal influenza vaccine

V3 = trivalent vaccine, H = 2009 H1N1, P=placebo

H1N1+Placebo/H1N1+Placebo/IIV3 = HP/HP/V3; H1N1+IIV3/H1N1+Placebo/Placebo = HV3/HP/P; H1N1+Placebo/H1N1+IIV3/Placebo = HP/HV3/P; and IIV3+Placebo/H1N1+Placebo/H1N1= V3P/HP/H

Table 4.c
Geometric Mean Titers (95% Confidence Intervals) as Assessed by the Hemagglutination Inhibition Assay, IIV3 H3 Antigen

All Subjects		Day 0		Day 21		Day 42		Day 63	
Study Group	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N
HP/HP/V3	199	47.0 (37.6- 58.6)	199	47.9 (38.4- 59.9)	189	55.6 (44.3- 69.9)	183	168.1 (138.8- 203.5)	
HV3/HP/P	198	56.0 (44.4- 70.5)	198	223.9 (185.1- 270.8)	184	216.3 (179.6- 260.4)	176	163.8 (134.5- 199.6)	
HP/HV3/P	200	43.8 (35.6- 53.8)	200	45.6 (37.1- 56.1)	183	225.0 (187.5- 270.0)	178	163.8 (134.4- 199.6)	
V3P/HP/HP	196	47.6 (38.6- 58.6)	196	222.3 (181.9- 271.8)	188	227.9 (190.3- 273.1)	181	165.6 (135.0- 203.2)	
Subjects Age 18 – 64 Years									
HP/HP/V3	98	42.9 (31.1- 59.2)	98	45.4 (32.6- 63.4)	94	54.9 (39.1- 77.2)	91	199.6 (151.6- 262.6)	
HV3/HP/P	101	40.6 (28.9- 56.9)	101	217.9 (162.5- 292.1)	92	206.7 (156.4- 273.2)	89	165.1 (122.4- 222.7)	
HP/HV3/P	99	30.4 (22.6- 40.9)	99	31.5 (23.5- 42.3)	90	235.2 (177.8- 311.0)	88	170.4 (126.4- 229.7)	
V3P/HP/HP	99	43.2 (32.3- 57.8)	99	264.9 (197.0- 356.2)	94	286.5 (224.9- 365.0)	90	217.7 (164.3- 288.5)	
Subjects Age 65 Years and Older									
HP/HP/V3	101	51.2 (37.6- 69.8)	101	50.5 (37.3- 68.4)	95	56.4 (41.3- 76.9)	92	141.8 (108.6- 185.3)	
HV3/HP/P	97	78.3 (57.7- 106.3)	97	230.4 (180.2- 294.4)	92	226.3 (176.1- 290.7)	87	162.6 (125.0- 211.4)	
HP/HV3/P	101	62.5 (47.6- 82.0)	101	65.6 (49.6- 86.6)	93	215.6 (169.5- 274.1)	90	157.6 (120.7- 205.7)	
V3P/HP/HP	97	52.5 (38.7- 71.1)	97	185.9 (141.7- 243.9)	94	181.4 (139.3- 236.2)	91	126.3 (94.4- 169.1)	

V3 = trivalent vaccine, H = 2009 H1N1, P=placebo

H1N1 +Placebo/H1N1 +Placebo/IIV3 = HP/HP/V3; H1N1 +IIV3/H1N1 +Placebo/Placebo = HV3/HP/P; H1N1 +Placebo/H1N1 +IIV3/Placebo = HP/HV3/P; and IIV3 +Placebo/H1N1 +Placebo/H1N1 = V3P/HP/H

Table 4.b
Geometric Mean Titers (95% Confidence Intervals) as Assessed by the Hemagglutination Inhibition Assay, IIV3 B Antigen

All Subjects*		Day 0		Day 21		Day 42		Day 63	
Study Group	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N
HP/HP/V3	199	33.8 (29.5- 38.8)	199	34.6 (30.0- 39.8)	189	32.5 (28.2- 37.3)	183	65.4 (57.0- 75.2)	
HV3/HP/P	198	38.9 (33.8- 44.8)	198	101.5 (86.1- 119.6)	184	82.4 (70.6- 96.3)	176	70.5 (60.9- 81.6)	
HP/HV3/P	200	35.3 (30.7- 40.7)	200	38.5 (33.4- 44.3)	183	82.5 (70.5- 96.5)	178	69.3 (60.0- 79.9)	
V3P/HP/HP	196	31.8 (27.8- 36.3)	196	89.9 (76.4- 105.8)	188	81.8 (69.6- 96.1)	181	62.6 (54.0- 72.6)	
Subjects Age 18 – 64 Years									
HP/HP/V3	98	31.5 (25.6- 38.6)	98	31.5 (25.5- 38.8)	94	31.6 (25.9- 38.5)	91	78.8 (65.2- 95.2)	
HV3/HP/P	101	34.6 (28.8- 41.6)	101	123.3 (99.5- 152.8)	92	98.0 (79.2- 121.4)	89	80.0 (64.7- 98.9)	
HP/HV3/P	99	33.1 (27.1- 40.4)	99	37.0 (30.4- 45.2)	90	103.9 (83.6- 129.3)	88	81.9 (66.9- 100.3)	
V3P/HP/HP	99	30.4 (25.3- 36.7)	99	135.3 (109.1- 167.6)	94	123.6 (100.1- 152.7)	90	87.1 (71.0- 106.8)	
Subjects Age 65 Years and Older									
HP/HP/V3	101	36.3 (30.1- 43.8)	101	37.9 (31.3- 45.8)	95	33.3 (27.3- 40.7)	92	54.5 (44.6- 66.5)	
HV3/HP/P	97	43.9 (35.3- 54.5)	97	82.9 (64.7- 106.3)	92	69.3 (55.4- 86.8)	87	62.0 (50.7- 75.8)	
HP/HV3/P	101	37.6 (30.7- 46.1)	101	40.0 (32.6- 49.0)	93	65.9 (52.9- 82.1)	90	58.8 (48.2- 71.8)	
V3P/HP/HP	97	33.2 (27.3- 40.4)	97	59.3 (47.6- 73.8)	94	54.1 (43.7- 67.1)	91	45.2 (37.2- 54.9)	

* Slight differences arise between the tables containing all per protocol subjects and the numbers quoted in the text that are a result of statistical modeling that drops 10 subjects who did not know if they had received the prior year's seasonal influenza vaccine

V3 = trivalent vaccine, H = 2009 H1N1, P=placebo

H1N1+Placebo/H1N1+Placebo/IIV3 = HP/HP/V3; H1N1+IIV3/H1N1+Placebo/Placebo = HV3/HP/P; H1N1+Placebo/H1N1+IIV3/Placebo = HP/HV3/P; and IIV3+Placebo/H1N1+Placebo/H1N1= V3P/HP/H