ORIGINAL ARTICLE

Efficacy of Recombinant Influenza Vaccine in Adults 50 Years of Age or Older

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ABSTRACT

BACKGROUND

Improved influenza vaccines are needed to control seasonal epidemics. This trial compared the protective efficacy in older adults of a quadrivalent, recombinant influenza vaccine (RIV4) with a standard-dose, egg-grown, quadrivalent, inactivated influenza vaccine (IIV4) during the A/H3N2-predominant 2014–2015 influenza season, when antigenic mismatch between circulating and vaccine influenza strains resulted in the reduced effectiveness of many licensed vaccines.

METHODS

We conducted a randomized, double-blind, multicenter trial of RIV4 (45 μg of recombinant hemagglutinin [HA] per strain, 180 μg of protein per dose) versus standard-dose IIV4 (15 μg of HA per strain, 60 μg of protein per dose) to compare the relative vaccine efficacy against reverse-transcriptase polymerase-chain-reaction (RT-PCR)–confirmed, protocol-defined, influenza-like illness caused by any influenza strain starting 14 days or more after vaccination in adults who were 50 years of age or older. The diagnosis of influenza infection was confirmed by means of RT-PCR assay and culture of nasopharyngeal swabs obtained from participants with symptoms of an influenza-like illness. The primary end point was RT-PCR–confirmed, protocol defined, influenza-like illness between 14 days or more after vaccination and the end of the influenza season.

RESULTS

A total of 9003 participants were enrolled and underwent randomization; 8855 (98.4%) received a trial vaccine and underwent an efficacy follow-up (the modified intention-to-treat population), and 8604 (95.6%) completed the per-protocol follow-up (the modified per-protocol population). Among RIV4 recipients, the RT-PCR–confirmed influenza attack rate was 2.2% (96 cases among 4303 participants) in the modified per-protocol population and 2.2% (96 cases among 4427 participants) in the modified intention-to-treat population. Among IIV4 recipients, the attack rate was 3.2% (138 cases among 4301 participants) in the modified per-protocol population and 3.1% (138 cases among 4428 participants) in the modified intention-to-treat population. A total of 181 cases of influenza A/H3N2, 47 cases of influenza B, and 6 cases of nonsubtypeable influenza A were detected. The probability of influenza-like illness was 30% lower with RIV4 than with IIV4 (95% confidence interval, 10 to 47; P=0.006) and satisfied prespecified criteria for the primary noninferiority analysis and an exploratory superiority analysis of RIV4 over IIV4. The safety profiles of the vaccines were similar.

CONCLUSIONS

RIV4 provided better protection than standard-dose IIV4 against confirmed influenza-like illness among older adults. (Funded by Protein Sciences; ClinicalTrials.gov number, NCT02285998.)

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Reducing the burden of influenza disease requires improved vaccines, and a recombinant influenza vaccine may contribute to this public-health goal.[1] This vaccine contains recombinant hemagglutinin (HA) proteins produced in a serum-free medium by *expres*SF+ cells. These cells contain recombinant baculovirus vectors carrying genes that code for HA. The process yields recombinant HA that is genetically identical to the selected influenza strains without extraneous egg proteins, formaldehyde, antibiotics, or preservatives. Influenza viruses are grown in eggs to produce the inactivated influenza vaccine (IIV); these viruses typically contain mutations in the genes that code for HA that may reduce vaccine effectiveness.[2-5] Recombinant techniques can be used to produce vaccine within 6 to 8 weeks instead of 6 months with the egg-grown process, and research on the incorporation of additional protective antigens in these vaccines is under way.[6-8]

Circulation of predominantly influenza A subtype H3N2 viruses that were antigenically mismatched to the vaccine strain in 2014–2015 resulted in an estimated seasonal vaccine effectiveness of 27 to 36% (adjusted) in adults 50 years of age or older[9,10] (an effectiveness that was lower than usual) and influenza-associated hospitalization rates among adults 65 years of age or older that were higher than usual.[10] During the 2014–2015 season, we conducted a randomized trial comparing quadrivalent, recombinant influenza vaccine (RIV4) with an egg-grown quadrivalent, inactivated influenza vaccine (IIV4) to assess the relative vaccine efficacy against reverse-transcriptase polymerase-chain-reaction (RT-PCR)–confirmed influenza-like illness. The primary hypothesis was that the efficacy of RIV4 would be noninferior relative to that of IIV4; an exploratory criterion for superiority was prespecified.

Methods

Trial Design and Oversight

We conducted a phase 3–4, randomized, double-blind, active-controlled trial comparing RIV4 with IIV4 in persons 50 years of age or older at 40 outpatient centers across the United States from October 22, 2014, through May 22, 2015. The trial was approved and monitored by an institutional review board (Quorum Review IRB) and was conducted in accordance with international standards.[11] The protocol information was registered with ClinicalTrials.gov on October 7, 2014, but the posting was released on October 27, 2014, because of a delay in the Protocol Registration and Results System. All participants provided written informed consent before any trial procedures were performed.

The authors had primary responsibility for the trial design and protocol development; the contract research organization (INC Research) for trial monitoring, data management, and statistical analyses; and the investigators at the trial centers for critical protocol review, trial procedures, and data collection.[12] All the authors assume responsibility for the accuracy and completeness of the data and for the fidelity of the trial to the protocol, which is available with the full text of this article at NEJM.org.

Participants and Group Assignments

Adults 50 years of age or older who were living independently without clinically significant acute illness, who were not receiving ongoing immunosuppressive therapy, and who had no contraindications to trial vaccines were stratified according to age (50 to 64 years vs. ≥65 years) and randomly assigned to receive a single dose of either RIV4 or IIV4. Treatments were assigned centrally with the use of an interactive voice-response system that assigned patients on the basis of computer-generated block randomization.

Participants, investigators, the trial sponsor, and trial staff remained unaware of the treatment assignments until trial completion and database lock. Personnel at each site who were aware of the treatment assignments obtained treatment assignments and prepared and administered the trial vaccine, but they did not evaluate the trial participants. Participants at five sites provided HA-inhibition serologic samples before and 28 days after vaccination.

Vaccines

RIV4 (Flublok Quadrivalent, Protein Sciences) contained 45 μg of recombinant HA per strain (180 μg of protein per dose). This vaccine was approved by the Food and Drug Administration (FDA) on October 7, 2016. IIV4, an FDA-approved inactivated vaccine (Fluarix Quadrivalent, GlaxoSmithKline), contained 15 μg of HA per strain (60 μg of protein per dose).

RIV4 was produced with the use of recombinant DNA techniques.[1] IIV4 was produced with the use of standard techniques for inactivating

and purifying infectious virus grown in eggs.[13] Both vaccines contained HAs of the strains recommended for the 2014–2015 season: A/California/7/2009 (H1N1)-like, A/Texas/50/2012 (H3N2), B/Massachusetts/2/2012, and B/Brisbane/60/2008. The vaccines were provided in prefilled 0.5-ml syringes and administered intramuscularly.

SURVEILLANCE AND ASCERTAINMENT OF INFLUENZA

After vaccination, participants called the interactive voice-response system twice weekly by telephone to report any respiratory symptoms (sore throat, cough, sputum production, wheezing, or difficulty breathing) or systemic symptoms such as fever (oral body temperature >37.2°C), chills, fatigue, headache, or myalgia. Participants with symptoms in either category were instructed to return to their trial site for influenza testing. The trial sites and the contract research organization were notified twice weekly of participants with symptoms of influenza-like illness or missing calls to the interactive voice-response system, and the trial personnel contacted participants every 2 weeks. Participants' calls to the interactive voice-response system continued until April 20, 2015, when the Centers for Disease Control and Prevention (CDC) reported influenza rates below threshold levels.[14]

An influenza-like illness was defined in the protocol as at least one symptom in both the respiratory and systemic illness categories, regardless of severity. Symptoms that met those criteria prompted collection of a nasopharyngeal swab at the trial site within 72 hours after the onset of disease. Influenza infection was confirmed by means of a validated 5-plex PCR assay (Focus Diagnostics) that detected the seasonal influenza types A or B and the A subtypes seasonal H1, H3, or pandemic H1, but not B lineages. PCR-positive samples were cultured in Madin–Darby Canine Kidney cells. Further antigenic characterization was not performed.

EFFICACY

The primary end point was RT-PCR–confirmed, protocol-defined, influenza-like illness that occurred between 14 days or more after vaccination and the end of the influenza season and was caused by any influenza virus type or subtype. Secondary efficacy end points included culture-positive influenza-like illness and RT-PCR–positive or culture-positive influenza-like illness with fever (body temperature ≥37.8°C). Widespread circulation of antigenically mismatched influenza A/H3N2 during the trial prompted separate post hoc analyses of efficacy against influenza A and influenza B. HA-inhibition antibody immunogenicity is described and shown in Fig. S1 in the Supplementary Appendix, available at NEJM.org.

SAFETY

Reports of local and systemic reactogenicity were solicited with the use of memory aids (e.g., diary cards) during the week after vaccination. Reactogenicity populations were defined as participants who did not have missing data in any of the three categories of solicited reports of local, systemic, or temperature reactions. All unsolicited reported adverse events were recorded for 28 days, and serious or medically attended adverse events were recorded for up to 6 months after vaccination; the last participant contact occurred on May 22, 2015.

STATISTICAL ANALYSIS

The sample size required to provide 80% power to show the noninferiority of relative vaccine efficacy was 4257 participants per treatment group, assuming influenza attack rates of 1.6% in the RIV4 group and 2.0% in the IIV4 group.[15] These assumptions were based on trials comparing trivalent, recombinant influenza vaccine (RIV3) with trivalent, inactivated influenza vaccine (IIV3), particularly against A/H3N2.[5,16,17] Noninferiority would be concluded if the lower bound of the 95% confidence interval for relative vaccine efficacy was greater than –20%. A total of 9000 participants (approximately 4500 per treatment group) allowed 4 to 5% loss to follow-up. Data on participants who were lost to follow-up were censored at the last trial contact. The prespecified criterion for superiority of RIV4 required a lower bound of the 95% confidence interval for relative vaccine efficacy greater than 9%, similar to the criterion in a previous registration trial of inactivated influenza vaccine.[18]

The efficacy of RIV4 relative to IIV4 was calculated as $100 \times (1 - \mathrm{RR})$, where RR is the ratio (relative risk) of influenza attack rates in the two groups, without respect to the timing of the onset of influenza. The confidence interval was calculated with the use of the Farrington–Manning score method for binomial proportions.[19]

With FDA concurrence, the primary analysis involved a modified per-protocol population including all participants who received trial vaccine

and provided efficacy data at least 14 days later with no major protocol deviations. A post hoc analysis of a modified intention-to-treat population included all randomly assigned participants who received trial vaccine and provided follow-up efficacy data at least 14 days later. For both efficacy and safety, the modified per-protocol population was evaluated according to the vaccine

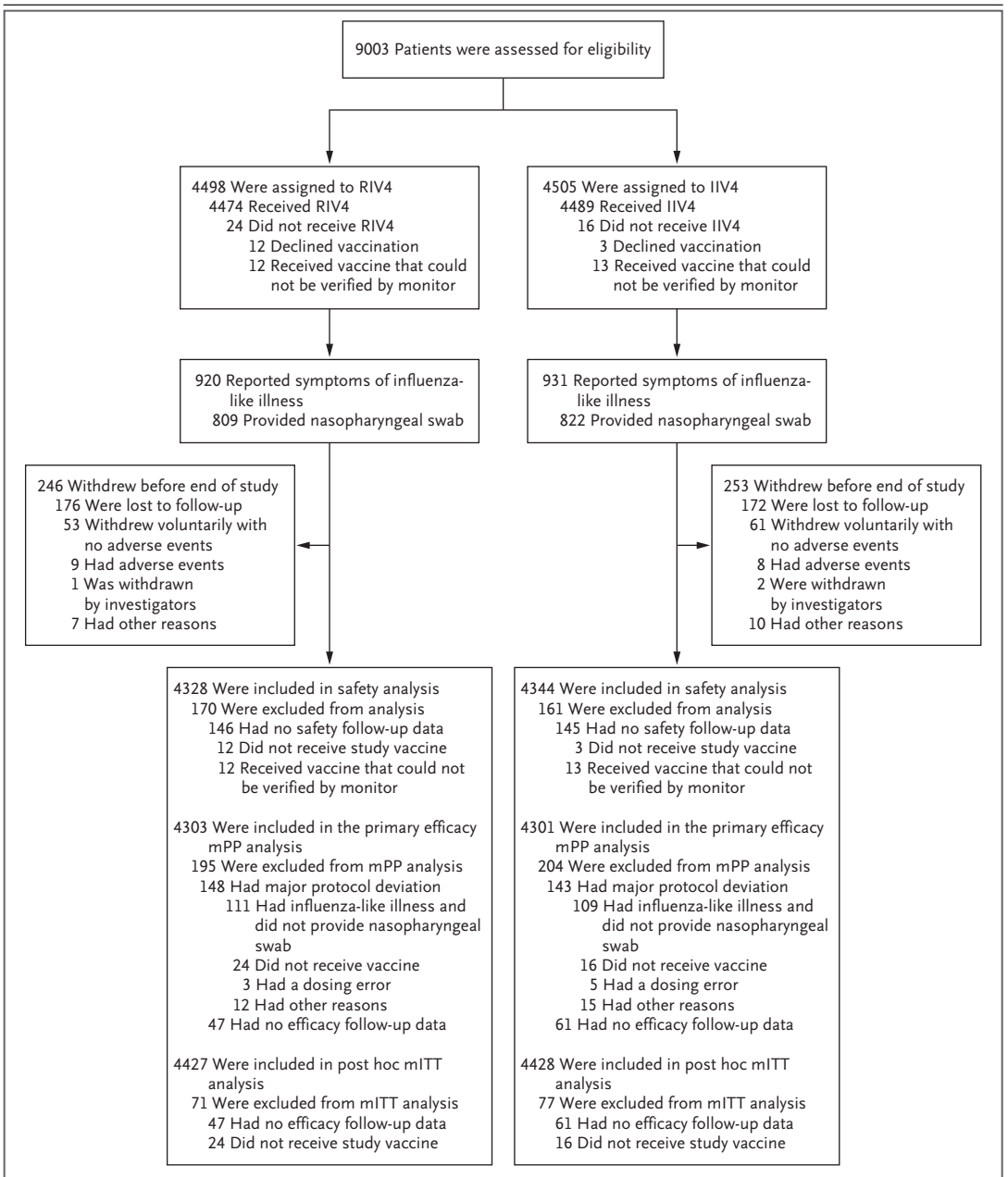

Figure 1. Screening, Enrollment, and Follow-up.

The number of participants in the safety population equaled the number of participants assigned to the treatment group minus the number excluded from the analysis. The number of participants in the modified per-protocol (mPP) population equaled the number of participants assigned to the treatment group minus the number excluded from the mPP analysis. The number of participants in the post hoc modified intention-to-treat (mITT) population equaled the number of participants assigned to the treatment group minus the number excluded from the mITT analysis. Participants may have had more than one protocol deviation. RIV4 denotes quadrivalent, recombinant influenza vaccine, and IIV4 quadrivalent, inactivated influenza vaccine.

received, regardless of treatment assignment; to assess the consistency of the results, key end points were reviewed with respect to prespecified subgroups of age (50 to 64 years vs. ≥65 years), sex, race, and ethnic group, and infection with influenza type A or B.

A Cox proportional-hazards model and log-rank test for significance were used to calculate hazard ratios for the development of influenza. The trial was not powered to draw independent conclusions according to subgroups; no adjustment for multiplicity was performed.[20] Reactogenicity analyses were adjusted for multiple comparisons with the use of a Bonferroni correction.[21] Statistical analyses were performed with the use of SAS software, version 9.3 or higher (SAS Institute).

RESULTS

PARTICIPANTS

A total of 9003 participants were enrolled between October 22 and December 22, 2014; 4498 were assigned to RIV4 and 4505 were assigned to IIV4. A total of 15 participants who underwent randomization withdrew consent before vaccination, 4474 received RIV4, and 4489 received IIV4 (Fig. 1). The type of vaccine administered to 25 additional participants at one site could not be verified, and these participants were excluded from all analyses, leaving 8963 in the full analysis population. A total of 8672 participants (96.8%) with post-vaccination data composed the safety population: 8604 of 8991 participants (95.7%) in

Table 1. Baseline Demographic and Coexisting Conditions of the RIV4 and IIV4 Vaccine Groups.*

Characteristic	RIV4 (N=4328)	IIV4 (N=4344)
Age — yr		
Mean	63	63
Range	50–96	50–94
Age group — no. (%)		
50–64 yr	2569 (59.4)	2617 (60.2)
≥65 yr	1759 (40.6)	1727 (39.8)
65–74 yr	1234 (28.5)	1254 (28.9)
≥75 yr	525 (12.1)	473 (10.9)
Male sex — no. (%)	1796 (41.5)	1807 (41.6)
Race or ethnic group — no. (%)†		
Black	773 (17.9)	753 (17.3)
White	3467 (80.1)	3493 (80.4)
Other	88 (2.0)	98 (2.3)
Hispanic ethnic group — no. (%)‡		
Hispanic	206 (4.8)	219 (5.0)
Non-Hispanic	4122 (95.2)	4123 (94.9)
Other	0	2 (<1)
Coexisting conditions — no. (%)		
Diabetes mellitus		
Insulin-dependent	170 (3.9)	176 (4.1)
Non–insulin-dependent	469 (10.8)	465 (10.7)
Atherosclerotic cardiovascular disease	1320 (30.5)	1318 (30.3)
Condition requiring statin lipid-lowering therapy	1194 (27.6)	1204 (27.7)
Condition requiring thiazide diuretic	332 (7.7)	340 (7.8)
Chronic obstructive pulmonary disease	144 (3.3)	151 (3.5)
Acid reflux or peptic ulcer disease	629 (14.5)	675 (15.5)
Depression	788 (18.2)	800 (18.4)

* There were no significant differences between the treatment groups. IIV4 denotes quadrivalent, inactivated influenza vaccine, and RIV4 quadrivalent, recombinant influenza vaccine.
† Race and ethnic group were reported by the participants. The category "Other" includes American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, and Asian.
‡ Some participants reported being neither Hispanic nor non-Hispanic.

the modified per-protocol efficacy population and 8855 of 8963 participants (98.8%) in the post hoc modified intention-to-treat population. The baseline characteristics and coexisting conditions of the participants in the two vaccine groups were similar (Table 1).

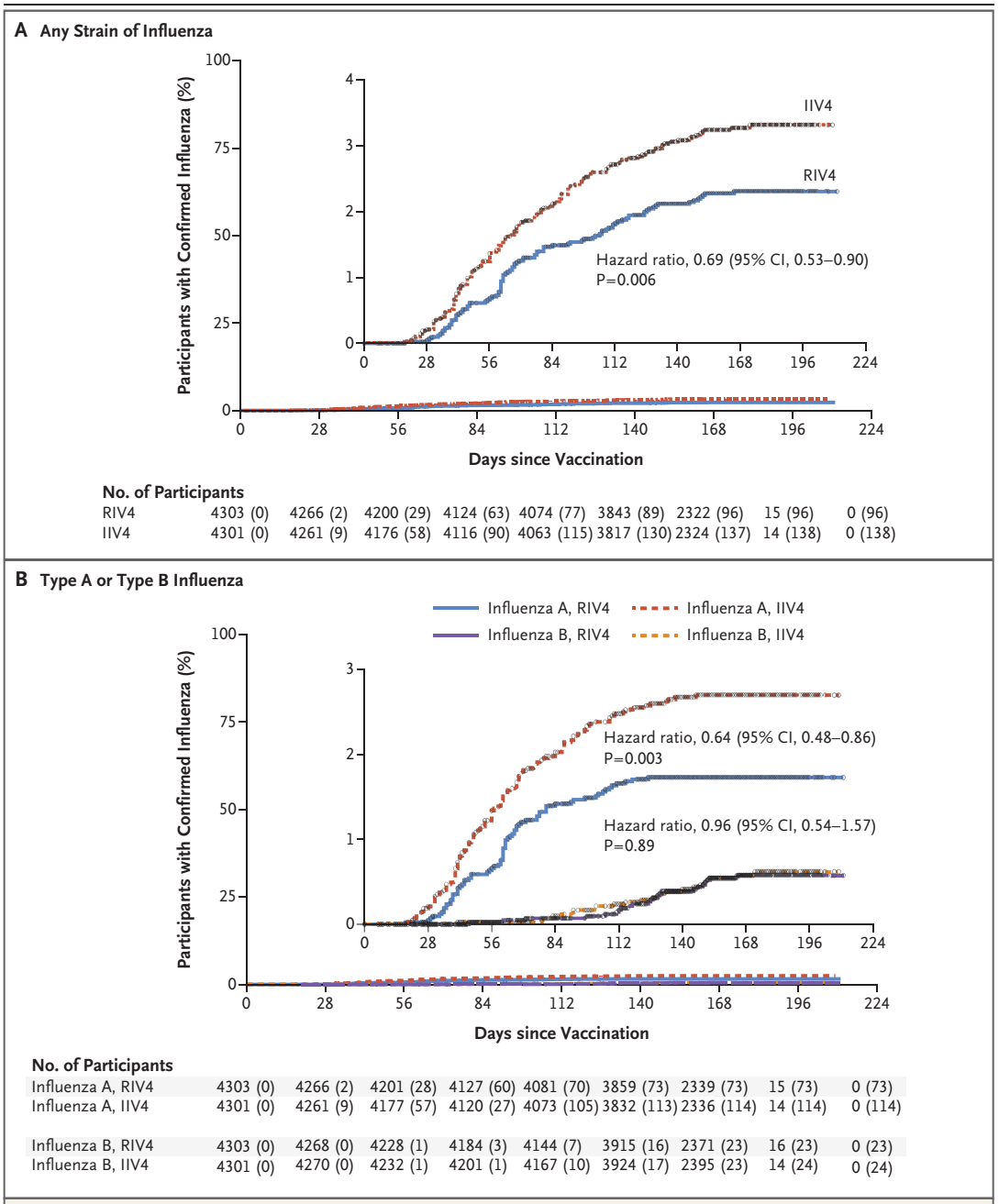

Figure 2. Confirmed Cases of Influenza-like Illness.

Confirmed protocol-defined, influenza-like illness was diagnosed by means of reverse-transcriptase–polymerase-chain-reaction assay. Panel A shows the cumulative incidence of influenza-like illness caused by any strain of influenza (primary analysis), and Panel B shows the cumulative incidence of influenza-like illness caused by type A influenza (upper pair of curves) or by type B influenza (lower pair of curves) (post hoc analysis). The insets show the same data on an enlarged y axis. CI denotes confidence interval.

ILLNESS SURVEILLANCE AND COLLECTION OF NASOPHARYNGEAL SWABS

To confirm the cause of protocol-defined influenza-like illnesses in the modified per-protocol population, nasopharyngeal swabs were obtained from 809 of 4328 recipients of RIV4 (18.7%) and from 822 of 4344 recipients of IIV4 (18.9%). In addition, 220 participants (111 RIV4 recipients and 109 IIV4 recipients) reported symptoms of influenza-like illness but did not provide a nasopharyngeal swab (a major protocol deviation). These participants were excluded from the primary modified per-protocol population analysis but were included in the post hoc modified intention-to-treat population analysis. Influenza was detected in 234 of 1631 samples (14.3%), including 181 cases of influenza A/H3N2, 47 cases of influenza B, and 6 cases of nonsubtypeable influenza A. No cases of influenza A/H1N1 were detected.

EFFICACY

The primary efficacy end point was confirmed in 96 of 4303 RIV4 recipients (2.2%) and in 138 of 4301 IIV4 recipients (3.2%); thus, in the modified per-protocol population, the probability of influenza-like illness was 30% lower with RIV4 than with IIV4 (95% confidence interval [CI], 10 to 47; P=0.006). In the post hoc efficacy analysis involving the modified intention-to-treat population, attack rates of 2.2% (96 cases among 4427 participants) and 3.1% (138 cases among 4428 participants) yielded essentially the same relative vaccine efficacy of 30% (95% CI, 10 to 47). The lower bound of the two-sided 95% confidence interval of 10% satisfied both the primary criterion for noninferiority and the prespecified exploratory superiority criterion. The cumulative incidence of RT-PCR–confirmed influenza-like illness showed significant efficacy of RIV4 over IIV4 throughout the influenza season (hazard ratio, 0.69; 95% CI, 0.53 to 0.90; P=0.006) (Fig. 2A). Post hoc analyses of relative vaccine efficacy against each influenza type showed a relative vaccine efficacy of RIV4 against influenza A of 36% (95% CI, 14 to 53) (hazard ratio, 0.64; 95% CI, 0.48 to 0.86; P=0.003) but no difference between the vaccines with respect to relative vaccine efficacy against influenza B (Figs. 2B and 3).

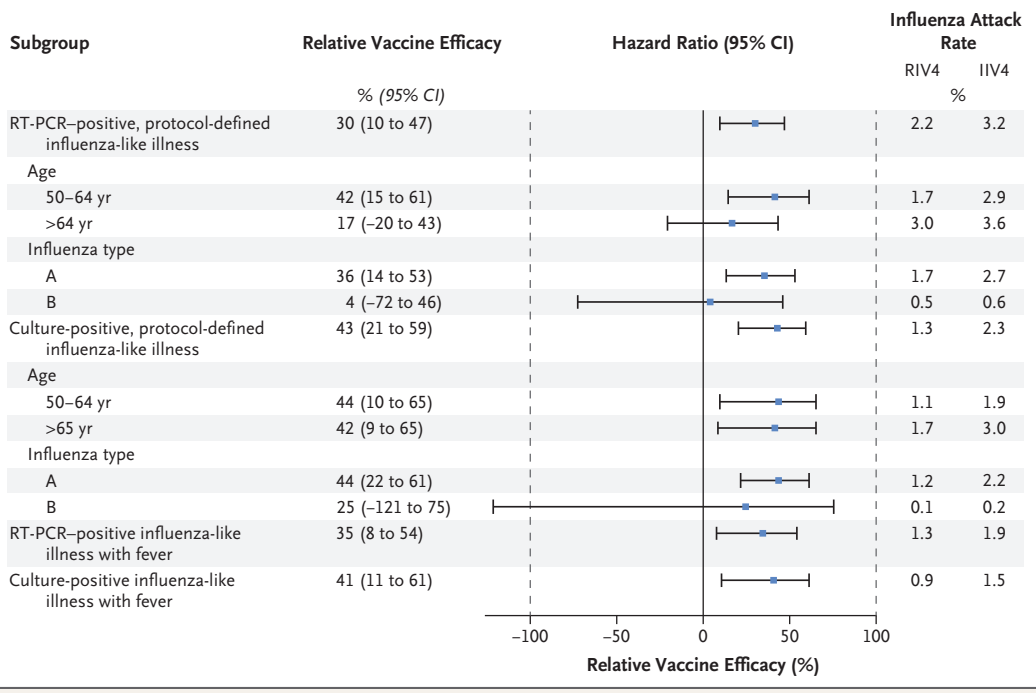

Figure 3. Relative Vaccine Efficacy in Various Population Subgroups.

The relative risk is the percentage of participants with documented flu in the RIV4 group (the RIV4 attack rate) divided by the percentage of participants with documented flu in the IIV4 group (the IIV4 attack rate). The relative vaccine efficacy was calculated as 100×(1−relative risk). RT-PCR denotes reverse-transcriptase polymerase chain reaction. The squares represent the point estimate of the treatment effect.

Table 2. Most Common Nonserious Unsolicited Adverse Events during Days 0 through 28.*

Condition	RIV4 (N=4328)	IIV4 (N=4344)
	no. of participants (%)	
Cough	226 (5.2)	253 (5.8)
Influenza-like illness	186 (4.3)	199 (4.6)
Oropharyngeal pain	178 (4.1)	177 (4.1)
Headache	143 (3.3)	145 (3.3)
Upper respiratory tract infection	129 (3.0)	156 (3.6)
Fatigue	106 (2.4)	100 (2.3)
Myalgia	95 (2.2)	79 (1.8)
Productive cough	59 (1.4)	97 (2.2)

* Events listed are those that occurred in 2% of participants or more in either treatment group.

Virus cultures of RT-PCR–positive nasopharyngeal swabs yielded fewer positive samples, but a higher relative vaccine efficacy of RIV4, than cultures of the same samples that were previously shown to be positive on RT-PCR: 43% (95% CI, 21 to 59) for all strains, 44% (95% CI, 22 to 61) for type A, and 25% (95% CI, –121 to 75) for type B (Fig. 3). Overall, the point estimates of the relative vaccine efficacy of RIV4 were consistently positive across age subgroups, definitions of clinical illness, and methods of laboratory confirmation (Fig. 3). Immunogenicity results from the 614 participants in the immunogenicity subgroup also showed higher antibody responses to A/H3N2 in RIV4 recipients (see Fig. S1 in the Supplementary Appendix).

SAFETY

Within 6 months after vaccination, 145 RIV4 recipients (3.4%) and 132 IIV4 recipients (3.0%) had at least one serious adverse event. All were events common among older adults, and none were considered by the trial team to be related to a trial vaccine (see Table S7 in the Supplementary Appendix). One RIV4 recipient and 3 IIV4 recipients reported hospitalization for documented influenza A; none of these patients provided nasopharyngeal swabs for the efficacy analyses. Death, which was assessed by the investigators who were unaware of the vaccine assignments as being unrelated to the vaccine or to complications of influenza, occurred in 8 RIV4 recipients and 12 IIV4 recipients. Causes of death are listed in Table S8 in the Supplementary Appendix. Most nonserious, unsolicited reported adverse events were of mild-to-moderate severity, and none were considered by the trial team to be related to the trial vaccine. There was no imbalance between the two treatment groups with respect to the most common unsolicited adverse events (in ≥2% of participants) (Table 2, and Table S10 in the Supplementary Appendix). All serious adverse events and common adverse events are listed according to severity in Tables S10 and S11 in the Supplementary Appendix.

Diaries in which participants recorded body temperature and local and systemic reactions were returned by more than 95% of participants in the two treatment groups. The incidences of injection-site pain and tenderness were slightly higher among IIV4 recipients than among RIV4 recipients, but all symptoms were of mild-to-moderate severity in the two groups and were usually of less than 3 days' duration (Table 3).

DISCUSSION

This head-to-head comparison of the clinical efficacy of RIV4 versus a standard-dose, egg-grown IIV4 showed that RIV4 satisfied the criterion for noninferiority of relative vaccine efficacy against RT-PCR–confirmed influenza-like illness in adults who were 50 years of age or older. The influenza attack rate among IIV4 recipients was similar to that reported among standard-dose IIV recipients in a recent randomized, controlled trial; this similarity provides support for the acceptability of the active-controlled design.[18] Furthermore, the prespecified exploratory criterion for the superiority of RIV4 over IIV4 was met. Although the planned analysis of this trial involved a modified per-protocol population, inclusion of all randomly assigned and vaccinated participants in a post hoc modified intention-to-treat population analysis of efficacy yielded essentially the same results.

The CDC[10] estimated that the adjusted overall vaccine effectiveness in adults 50 years of age or older in 2014–2015 was 27 to 36%, with a 95% confidence interval that excluded zero. The positive relative vaccine efficacy of RIV4 for both PCR-confirmed and culture-confirmed influenza-like illness, each with a 95% confidence interval that excluded zero, is consistent with the clinical benefit of RIV4. Influenza A/H3N2 infections constituted approximately 80% of the influenza strains identified[9] in 2014–2015 and 80% of the

Table 3. Local Injection-Site Reactions and Fever within 7 Days after Vaccination.*

Symptom	RIV4 (N=4307)	IIV4 (N=4319)	P Value†
One or more local events — no. (%)	1621 (37.6)	1745 (40.4)	0.009
Injection-site pain — no. (%)	813 (18.9)	950 (22.0)	<0.001†
Injection-site tenderness — no. (%)	1479 (34.3)	1604 (37.1)	0.007†
Redness — no. (%)	122 (2.8)	87 (2.0)	0.014
Firmness or swelling — no. (%)	142 (3.3)	115 (2.7)	0.09
Fever — no./total no. (%)	19/4262 (0.4)	21/4282 (0.5)	0.87

* The reactogenicity population comprised all vaccinated participants who recorded reactogenicity data on the Memory Aid at least once within 7 days after vaccination.
† P values were deemed to be significant on the basis of adjustment for multiple comparisons with the use of the Bonferroni correction.[21]

strains identified in this trial; these findings suggest that the benefit of RIV4 is likely to be seen with RIV3.[22,23] Both vaccines performed similarly against influenza B viruses, for which the estimated effectiveness in 2014–2015 was 36 to 79%.[10] The 30% efficacy of RIV4 relative to IIV4 was driven by the efficacy against influenza A/H3N2.

These efficacy results against probably antigenically mismatched viruses are consistent with those of previous trials showing efficacy and antibody responses against A/H3N2.[16,24-28] The safety data are also consistent with those of previous studies, since the reactogenicity observed with the RIV4, despite a higher HA protein content, was similar to that of IIV4.

In other studies of recombinant HA and IIV vaccines, higher doses than the standard dose of 15 μg of HA have been associated with greater immunogenicity and improved efficacy.[18,29] Recombinant influenza vaccines that contain 45 μg of each antigen have been associated with greater immunogenicity than that of vaccines with less antigen, particularly against influenza A/H3N2 strains.[16,18,24,26,29] Mutations in the genes that code for HA (especially H3), which are induced by adaptation to growth in eggs, can reduce vaccine effectiveness.[2-5] The higher quantity and greater accessibility of the genetically conserved stalk region of recombinant HA produced in the cells of lepidopteran insects have been speculated to yield cross-protection against mismatched influenza strains.[30] It is uncertain whether a higher antigen content or genetic fidelity to the recommended strain was responsible for the better relative vaccine efficacy of RIV4 in this trial.

In this trial, the efficacy of RIV4 did not appear to be hampered by the absence of neuraminidase (NA), since the relative vaccine efficacy of RIV4 compared favorably with IIV4, although the quantity and functional integrity of NA in IIV4 are unknown. A recombinant protein vaccine containing both HA and a functional quantity of NA could be explored and may provide additional protection, especially when HA antigenic mismatch occurs between circulating and vaccine strains.[6,7,31]

This trial has several limitations. It provides an estimate of the efficacy of RIV4 relative to IIV4; therefore, absolute efficacy can only be inferred on the basis of epidemiologic surveillance. In addition, estimates of the efficacy of RIV4 against influenza types and for population subgroups, based on limited numbers of participants, may lack precision. The trial, conducted during a single influenza season, cannot address either efficacy against a variety of influenza types and subtypes or RIV4 efficacy in persons who are vaccinated annually over multiple years. Despite a lack of testing for antigenic similarity, CDC surveillance for 2014–2015 suggests that the trial influenza A strains were unlikely to have been antigenically similar to the vaccines.[9,10] Results might differ in seasons when circulating strains match the vaccines. Finally, the trial allowed enrollment of persons with coexisting conditions that commonly occur in older adults in the United States, but persons with acute illnesses and those with immunosuppressive conditions or those receiving immunosuppressive therapy were excluded. Extrapolation of the results of this trial to such persons should be done with caution.

In conclusion, this trial showed that RIV4, as compared with IIV4, improved protection against laboratory-confirmed influenza-like illness in adults 50 years of age or older. These results occurred during an influenza season characterized by widespread circulation of antigenically mismatched strains of influenza A/H3N2.

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Drs. Dunkle, Izikson, and Cox report being employed by and holding stock in Protein Sciences; Dr. Patriarca, receiving consulting fees from Altimmune, FluGen, Georgia Institute of Technology, Medicago, VaxInnate, Vaxart, Vivaldi Biosciences, Moderna Therapeutics, Novavax, Seqirus, and Visterra; and Dr. Goldenthal, receiving consulting fees from Pfizer, Johnson & Johnson, Novartis, and the Bill and Melinda Gates Foundation. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES

1. Cox MMJ, Hashimoto Y. A fast track influenza virus vaccine produced in insect cells. J Invertebr Pathol 2011;107:Suppl: S31-S41.
2. Katz JM, Webster RG. Efficacy of inactivated influenza A virus (H3N2) vaccines grown in mammalian cells or embryonated eggs. J Infect Dis 1989;160: 191-8.
3. Wang ML, Katz JM, Webster RG. Extensive heterogeneity in the hemagglutinin of egg-grown influenza viruses from different patients. Virology 1989;171:275-9.
4. Rajakumar A, Swierkosz EM, Schulze IT. Sequence of an influenza virus hemagglutinin determined directly from a clinical sample. Proc Natl Acad Sci U S A 1990; 87:4154-8.
5. Skowronski DM, Janjua NZ, De Serres G, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS One 2014;9(3):e92153.
6. Johansson BE, Cox MMJ. Influenza viral neuraminidase: the forgotten antigen. Expert Rev Vaccines 2011;10:1683-95.
7. Johansson BE, Matthews JT, Kilbourne ED. Supplementation of conventional influenza A vaccine with purified viral neuraminidase results in a balanced and broadened immune response. Vaccine 1998;16:1009-15.
8. Milián E, Kamen AA. Current and emerging cell culture manufacturing technologies for influenza vaccines. Biomed Res Int 2015;2015:504831.
9. Appiah GD, Blanton L, D'Mello T, et al. Influenza activity — United States, 2014–15 season and composition of the 2015–16 influenza vaccine. MMWR Morb Mortal Wkly Rep 2015;64:583-90.
10. Flannery B, Clippard J. End-of-season influenza vaccine effectiveness estimates for the 2014-15 season: US Influenza Vaccine Effectiveness (Flu VE) Network. Atlanta: Advisory Committee on Immunization Practices, June 24, 2015.
11. Guidance for industry: e6 good clinical practices; consolidated guidance. Silver Spring, MD: Food and Drug Administration, 1996 (https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm073122.pdf).
12. Davidoff F, DeAngelis CD, Drazen JM, et al. Sponsorship, authorship, and accountability. N Engl J Med 2001;345:825-7.
13. Gerdil C. The annual production cycle for influenza vaccine. Vaccine 2003;21: 1776-9.
14. National and regional level outpatient and virus surveillance: FluView. Atlanta: Centers for Disease Control and Prevention, 2015 (http://gis.cdc.gov/grasp/fluview/fluportaldashboard.html); Season: 2014–15; Viral Surveillance: N/A.
15. PASS 13 statistical software. Salt Lake City: NCSS, 2014.
16. Keitel WA, Treanor JJ, El Sahly HM, et al. Comparative immunogenicity of recombinant influenza hemagglutinin (rHA) and trivalent inactivated vaccine (TIV) among persons > or =65 years old. Vaccine 2009;28:379-85.
17. Flannery B, Thaker SN, Clippard J, et al. Interim estimates of 2013–14 seasonal influenza vaccine effectiveness — United States, February 2014. MMWR Morb Mortal Wkly Rep 2014;63:137-42.
18. DiazGranados CA, Dunning AJ, Kimmel M, et al. Efficacy of high-dose versus standard-dose influenza vaccine in older adults. N Engl J Med 2014;371:635-45.
19. Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Stat Med 1990;9:1447-54.
20. Wang R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in medicine — reporting of subgroup analyses in clinical trials. N Engl J Med 2007;357:2189-94.
21. Dunn OJ. Multiple comparisons among means. J Am Stat Assoc 1961;56:52-64.
22. Estimates of deaths associated with seasonal influenza — United States, 1976–2007. MMWR Morb Mortal Wkly Rep 2010;59:1057-62.
23. Simonsen L, Clarke MJ, Williamson GD, Stroup DF, Arden NH, Schonberger LB. The impact of influenza epidemics on mortality: introducing a severity index. Am J Public Health 1997;87:1944-50.
24. Treanor JJ, El Sahly HM, King, et al. Protective efficacy of a trivalent recombinant hemagglutinin protein vaccine (FluBlok) against culture-confirmed influenza in healthy adults: a randomized, placebo-controlled trial. Vaccine 2011;29:7733-9.
25. Cox MMJ, Karl Anderson D. Production of a novel influenza vaccine using insect cells: protection against drifted strains. Influenza Other Respir Viruses 2007;1:35-40.
26. Baxter R, Patriarca PA, Ensor K, Izikson R, Goldenthal KL, Cox MM. Evaluation of the safety, reactogenicity and immunogenicity of FluBlok trivalent recombinant baculovirus-expressed hemagglutinin influenza vaccine administered intramuscularly to healthy adults 50-64 years of age. Vaccine 2011;29:2272-8.
27. Safdar A, Rodriguez MA, Fayad LE, et al. Dose-related safety and immunogenicity of baculovirus-expressed trivalent influenza vaccine: a double-blind, controlled trial in adult patients with non-Hodgkin B cell lymphoma. J Infect Dis 2006;194: 1394-7.
28. Powers DC, Smith GE, Anderson EL, et al. Influenza A virus vaccines containing purified recombinant H3 hemagglutinin are well tolerated and induce protective immune responses in healthy adults. J Infect Dis 1995;171:1595-9.
29. Treanor JJ, Schiff GM, Hayden FG, et al. Safety and immunogenicity of a baculovirus-expressed hemagglutinin influenza vaccine: a randomized controlled trial. JAMA 2007;297:1577-82.
30. Nachbagauer R, Choi A, Izikson R, Cox MM, Palese P, Krammer F. Age dependence and isotype specificity of influenza virus hemagglutinin stalk-reactive antibodies in humans. MBio 2016;7(1):e01996-15.
31. Monto AS, Petrie JG, Cross RT, et al. Antibody to influenza virus neuraminidase: an independent correlate of protection. J Infect Dis 2015;212:1191-9.