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Comparison of the Immunogenicity of Cell Culture-Based and Recombinant Quadrivalent Influenza Vaccines to Conventional Egg-Based Quadrivalent Influenza Vaccines Among Healthcare Personnel Aged 18–64 Years: A Randomized Open-Label Trial

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Background. RIV4 and cell-culture based inactivated influenza vaccine (ccIIV4) have not been compared to egg-based IIV4 in healthcare personnel, a population with frequent influenza vaccination that may blunt vaccine immune responses over time. We conducted a randomized trial among healthcare personnel (HCP) aged 18–64 years to compare humoral immune responses to ccIIV4 and RIV4 to IIV4.

Methods. During the 2018–2019 season, participants were randomized to receive ccIIV4, RIV4, or IIV4 and had serum samples collected prevaccination, 1 and 6 months postvaccination. Serum samples were tested by hemagglutination inhibition (HI) for influenza A/H1N1, B/Yamagata, and B/Victoria and microneutralization (MN) for A/H3N2 against cell-grown vaccine reference viruses. Primary outcomes at 1 month were seroconversion rate (SCR), geometric mean titers (GMT), GMT ratio, and mean fold rise (MFR) in the intention-to-treat population.

Results. In total, 727 participants were included (283 ccIIV4, 202 RIV4, and 242 IIV4). At 1 month, responses to ccIIV4 were similar to IIV4 by SCR, GMT, GMT ratio, and MFR. RIV4 induced higher SCRs, GMTs, and MFRs than IIV4 against A/H1N1, A/H3N2, and B/Yamagata. The GMT ratio of RIV4 to egg-based vaccines was 1.5 (95% confidence interval [CI] 1.2–1.9) for A/H1N1, 3.0 (95% CI: 2.4–3.7) for A/H3N2, 1.1 (95% CI: .9–1.4) for B/Yamagata, and 1.1 (95% CI: .9–1.3) for B/Victoria. At 6 months, ccIIV4 recipients had similar GMTs to IIV4, whereas RIV4 recipients had higher GMTs against A/H3N2 and B/Yamagata.

Conclusions. RIV4 resulted in improved antibody responses by HI and MN compared to egg-based vaccines against 3 of 4 cell-grown vaccine strains 1 month postvaccination, suggesting a possible additional benefit from RIV4.

Keywords. influenza vaccines; immunogenicity; healthcare personnel; COVID-19; cohort studies.

Influenza is estimated to result in 9–45 million illnesses, 140 000–810 000 hospitalizations, and 12 000–61 000 deaths each season in the United States [1]. Observed influenza vaccine effectiveness has been lower against A/H3N2 viruses than A/H1N1 viruses during recent seasons in the United States [2–4] which is concerning because influenza A/H3N2 viruses have been associated with higher influenza-associated hospitalization and mortality rates among older adults [5, 6].

Mutations incurred during egg-based vaccine strain production may reduce vaccine effectiveness against influenza A/H3N2 viruses in some seasons [7–9]. The conventional method of inactivated influenza vaccine (IIV) production relies on propagation in embryonated chicken eggs of a vaccine seed strain derived from a circulating influenza virus. Serial passage of influenza viruses in chicken eggs can result in mutations that cause important antigenic differences between the vaccine strain and circulating wild-type strains [7, 10–12]. Historically, the immunogenicity of influenza vaccines was assessed by measuring antibody responses to egg-grown influenza viruses, which may be a suboptimal measure of efficacy if egg-grown viruses differ antigenically from circulating wild-type viruses.

Vaccine strains that do not rely on egg-based production may induce higher immune responses to circulating

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influenza strains than egg-based vaccines [7]. During the past decade, a cell culture-based influenza vaccine (Flucelvax Quadrivalent™ by Seqirus, Inc., ccIIV4) and a recombinant influenza vaccine (Flublok Quadrivalent® by Sanofi Pasteur, RIV4) were licensed for use in the United States. RIV4 has a higher hemagglutinin (HA) content (45 µg of HA per strain) than standard-dose IIV4 and ccIIV4 (both 15 µg of hemagglutinin [HA] per strain) but does not contain any neuraminidase (NA) antigen. In contrast, both ccIIV4 and IIV4 contain varying amounts of NA. Prelicensure trials evaluating these vaccines measured antibody responses to a variety of targets including egg-grown viruses, cell-grown viruses, and baculovirus expression vector systems (BEVS)-derived antigen [13–16]. Although several recent trials have documented improved humoral immune responses to RIV4 compared to IIV4 in adults 18–64 years [17] and ≥65 years of age [18, 19], RIV4 and ccIIV4 have not been evaluated against IIV4 in highly influenza-vaccinated working-age adult populations in whom immune responses to influenza vaccination may be blunted over time [20]. To date, there are few data directly comparing the immunogenicity of cell-based and recombinant influenza vaccines to egg-based vaccines using the same immunogenicity outcome measures against the same antigenic targets.

This randomized, open-label trial assessed humoral immune responses to ccIIV4 and RIV4 compared to egg-based standard dose IIVs (Fluarix Quadrivalent™, GlaxoSmithKline; and Fluzone Quadrivalent™, Sanofi Pasteur) among United States (US) healthcare personnel (HCP) aged 18–64 years using cell-grown vaccine reference viruses. Because multiple egg-based IIVs with varying non-HA components such as NA and preservatives are available in the United States, 2 egg-based standard dose IIVs were combined as a single comparator group to improve generalizability of results. The primary study hypothesis was that a single dose of ccIIV4 or RIV4 would induce comparable or higher antibody titers against cell-grown vaccine viruses than a single dose of egg-based influenza vaccine in HCP with frequent prior influenza vaccination.

METHODS

Trial Design and Participants

This study was a randomized, open-label trial conducted at 2 sites during the Northern Hemisphere 2018–2019 and 2019–2020 influenza seasons. Study sites included 2 integrated healthcare systems: Baylor Scott & White Health (BSWH) in Temple, Texas, and Kaiser Permanente Northwest (KPNW) in Portland, Oregon. An open-label design was used because documentation of influenza vaccination receipt (including vaccine type) was a requirement for HCP at both health systems. HCP aged 18–64 years were enrolled during September–October. Results from the first year of the trial are described here. See Supplementary Methods for recruitment procedures and eligibility criteria.

Randomization and Blinding

Both participants and study investigators were aware of study arm assignments. Laboratory investigators were blinded to assignment until testing was completed. Enrolled HCP stratified by age groups (18–44 years and 45–64 years) were assigned to receive ccIIV4, RIV4, Fluzone IIV4, or Fluarix IIV4 using a site-stratified REDCap-based randomization system (see Supplementary Methods for details).

Intervention

At enrollment, randomized HCP received a 0.5-mL dose of study vaccine via intramuscular injection into the deltoid muscle of the upper arm. All 4 study vaccines contained antigens representative of the recommended 2018–2019 Northern Hemisphere influenza vaccine strain composition: an A/Michigan/45/2015 (H1N1)pdm09-like virus; an A/Singapore/INFIMH-16–0019/2016 (H3N2)-like virus; a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage); and a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage). During the 2018–2019 influenza season, egg-based IIV4 were produced from egg-derived seed viruses (or viral isolates), ccIIV4 contained cell culture derived H3N2 and B seed viruses and egg-derived H1N1 seed virus, and RIV4 contained recombinant HA proteins based on cell-culture derived seed viruses.

Study Procedures

At enrollment, eligible and consented HCP had 20 mL of venous blood drawn for serologic assays. HCP also completed online enrollment surveys and were asked to come back at approximately 1 and 6 months postvaccination for collection of 20 mL of venous blood at each visit. During the period of influenza circulation, sites conducted active surveillance for influenza-like illness (ILI) with mid-turbinate nasal swab collection and testing for influenza viruses. Surveillance was conducted to identify vaccine failures and not to assess clinical efficacy end points. See Supplementary Methods for additional details about ILI surveillance.

Outcomes Measures

The coprimary outcomes were serologic responses to cell-grown vaccine reference viruses by hemagglutination inhibition (HI) for influenza A/H1N1, influenza B/Yamagata, and influenza B/Victoria vaccine and by microneutralization (MN) assay to influenza A/H3N2 at approximately 1 month postvaccination using the following measures: seroconversion rate (SCR), geometric mean titers (GMT), mean fold rise (MFR), and geometric mean titer ratio. SCR was defined as the proportion of participants with either a prevaccination titer of <1:10 and 1 month postvaccination titer ≥1:40 or a prevaccination titer

≥1:10 and a ≥ 4-fold rise between pre- and postvaccination titers. MFR was defined as the geometric mean of the ratio of postvaccination titer and prevaccination titer for each subject. GMT ratio was defined as the ratio of postvaccination GMTs between either ccIIV4 or RIV4 compared to the egg-based vaccine group. Secondary outcomes were titers ≥1:40, 1:80, and 1:160 against cell-grown vaccine reference viruses by HI or MN at approximately 1 month postvaccination.

Subgroup analyses to evaluate for heterogeneity of effects among HCP stratified by number of influenza vaccines received during the preceding 5 years were prespecified in the study protocol.

Blood Specimen Testing

HI assays were performed using 0.5% turkey erythrocytes against the cell-grown A/Michigan/45/2015 (H1N1)pdm09, B/Colorado/06/2017; and B/Phuket/3073/2013 using methods that have been previously described [21]. Cell-grown A/H1N1 and B viruses were propagated in Madin-Darby-Canine-Kidney (MDCK) cells. All B antigens were ether treated prior to HI assays.

MN assays against cell-grown A/Singapore/INFIMH-16–0019/2016 (H3N2) as previously described [22]. Cell-grown A/Singapore/INFIMH-16–0019/2016 were propagated in MDCK-SIAT1 cells.

All viruses used in the study were sequenced and confirmed with no additional mutations compared to seed strains. A/H1N1 and A/H3N2 antigens were cultivated at the Centers for Disease Control and Prevention (CDC), and the B antigens were provided by Seqirus and then further ether treated at CDC. See Supplementary Methods for details about blood specimen collection and processing.

Sample Size

Assuming a Type 1 error of 5% and a Type 2 error of 20%, a minimum sample size of at least 696 with at least 203 participants in the ccIIV4 and RIV4 arms and 145 participants in each of the Fluzone IIV4 and Fluarix IIV4 arms was anticipated to provide adequate statistical power to detect a difference in postvaccination GMT of ≥2-fold between study arms if postvaccination GMT was ≥20 in the combined IIV4 arms and a relative difference in postvaccination SCR of 30% if the postvaccination SCR was ≥50% in the combined egg-based IIV4 arms.

Data Analysis

The full analytic intention-to-treat (ITT) population comprised randomized HCP meeting eligibility criteria regardless of vaccine receipt. The 1 month and 6 month per protocol populations comprised randomized HCP who received study vaccine and had serum samples drawn and tested at 1 month or 1 and 6 months postvaccination, respectively, within the protocol-specified acceptable time periods. Primary analyses for outcomes at 1 month postvaccination were ITT. Secondary analyses for outcomes at 6 months postvaccination were per protocol. To address missing data, a "worst-case scenario" analytic approach was used for ITT analyses in which a titer of 1:5 (ie, undetectable) was assigned for all missing data.

Participants in the Fluzone IIV4 and Fluarix IIV4 groups were initially evaluated separately for the primary endpoints of SCR and GMT at 1 month postvaccination using prespecified criteria to determine whether the 2 groups would be collapsed into a single comparator group (combined egg-based IIV4). The prespecified criteria were based on effect sizes for which there would be adequate statistical power to detect differences based on the goal sample size. Comparison of Fluzone IIV4 and Fluarix IIV4 participants met the prespecified criteria of <15% absolute difference in SCR and a ≤2-fold difference in postvaccination GMT between participants in the 2 groups (Supplementary Tables 1a, 1b, 2a, and 2b). Therefore, participants in both groups were combined into a single egg-based vaccine comparator group for subsequent analyses evaluating ccIIV4 and RIV4 recipients.

Frequencies of seroconversion and postvaccination HI and MN titers greater than prespecified cutoffs were compared between vaccine arms using χ^2 test. GMTs, GMT ratios, and MFR were compared using Student *t* test. All tests were 2-tailed with a level of significance of .05. See Supplementary Methods for details about prespecified subgroup analyses and post hoc analyses. Analyses were performed with SAS (Version 9.3) (SAS Institute, Cary, North Carolina, USA).

Ethical Review

The study protocol was reviewed and approved by the institutional review boards (IRBs) of the 2 study sites and Abt Associates, which provided site oversight and data management support. The IRB of the CDC relied upon the single IRB review of the BSWH IRB. This study is registered in ClinicalTrials.gov, number NCT03722589. Study findings are reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) statement guidelines.

RESULTS

Study Enrollment and Participant Baseline Characteristics

Overall, 952 HCP were assessed for eligibility, of whom 225 (24%) were excluded (Figure 1). The remaining 727 HCP were enrolled, randomized and included in the ITT population. Of these, all participants allocated to the Fluzone IIV4, Fluarix IIV4, and ccIIV4 arms received study vaccine, and 98% (198/202) allocated to the RIV4 arm received study vaccine. One and 6 month per protocol retention rates by vaccine arm were 90% and 70% for Fluzone IIV4, 98% and 81% for Fluarix IIV4, 99% and 86% for ccIIV4, and 97% and 82% for RIV4.

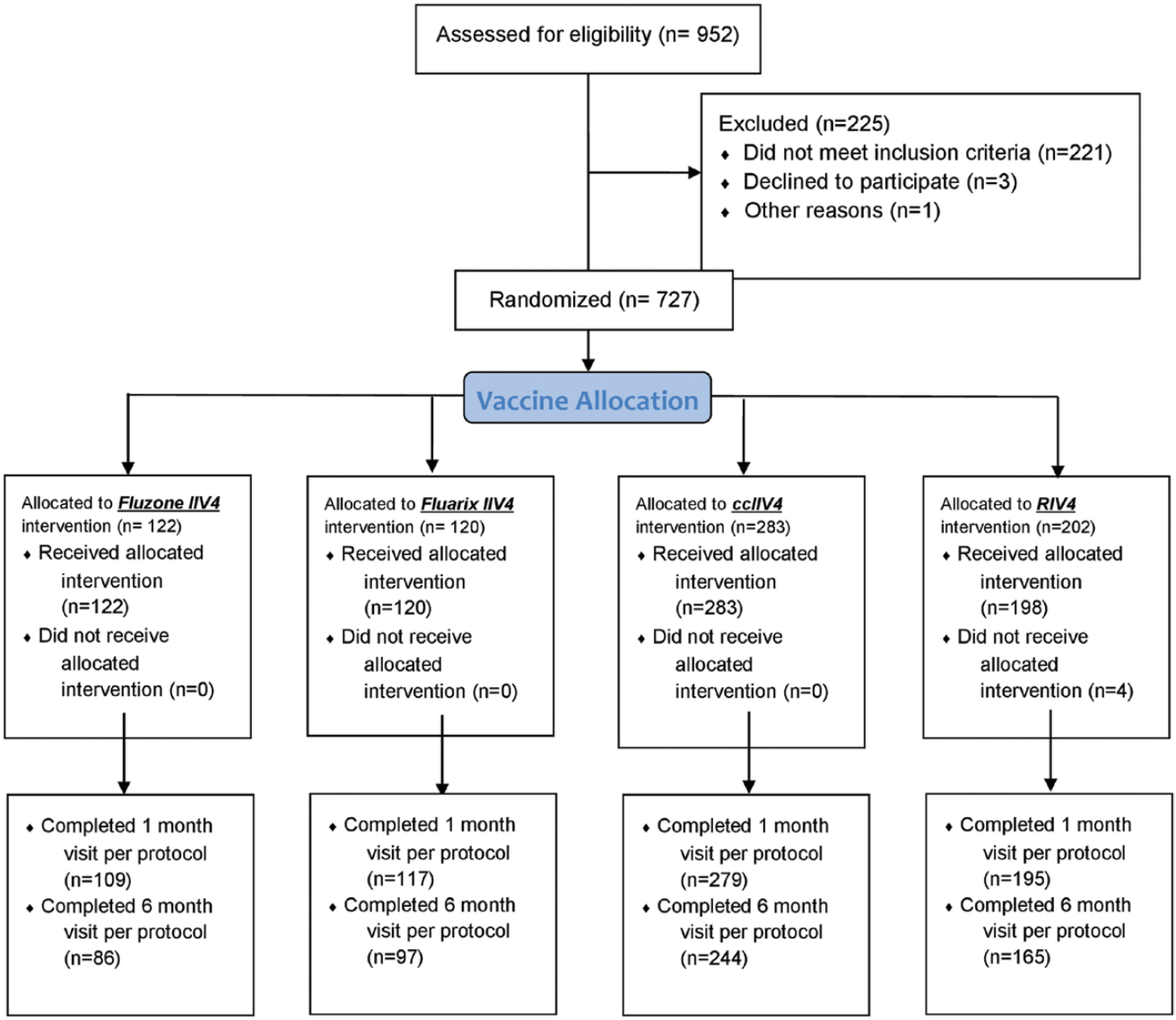

Figure 1. Screening, enrollment, and retention* in a randomized, open-label trial comparing the immunogenicity of cell culture-based and recombinant influenza vaccines to conventional egg-based vaccines among healthcare personnel aged 18–64 years, 2018–2019 influenza season. *For Fluzone IIV4, 12 participants were withdrawn from the study and did not have serum samples tested after they received vaccine that was left out at room temperature for an extended period; in addition, 1 participant completed the 1 month visit outside the per protocol time window; an additional 1 participant completed the 6 month visit per protocol but did not have a serum sample tested, 10 participants did not complete the 6 month visit, and 12 completed it outside the per protocol time window. For Fluarix IIV4, 1 participant did not complete the 1 month visit, 1 participant completed it outside the per protocol time window, and 1 completed it per protocol but did not have serum samples tested; an additional 11 participants did not complete the 6 month visit, and 9 participants completed it outside the per protocol time window. For ccIIV4, 2 participants did not complete the 1 month visit, 1 participant completed it outside the per protocol time window, and 1 completed it per protocol but did not have serum samples tested; an additional 14 participants did not complete the 6 month visit, and 21 participants completed it outside the per protocol time window. For RIV4, 2 participants did not complete the 1 month visit, and 1 participant completed it outside the per protocol time window; an additional 19 participants did not complete the 6 month visit, and 11 participants completed it outside the per protocol time window. Abbreviations: ccIIV4, cell-culture based IIV4 represented by Flucelvax Quadrivalent™ by Seqirus; IIV4, quadrivalent inactivated influenza vaccine represented by Fluzone by Sanofi Pasteur and Fluarix by GSK Biologicals; RIV4, recombinant IIV4 represented by Flublok by Sanofi Pasteur.

Among the ITT population, participants in each vaccine arm were similar with respect to age, sex, race, ethnicity, body mass index (BMI), and mean subjective health status score (Table 1). Participants in all study arms reported receiving an average of 5 influenza vaccines during the preceding 5 seasons; only 1–2% in each vaccine arm reported having never received an influenza vaccine during the preceding 5 seasons. Baseline geometric mean HI or MN titers were similar against vaccine reference viruses among participants in the combined IIV4, ccIIV4, and RIV4 arms (Table 2, Supplementary Table 3).

Antibody Responses at One Month Postvaccination

At 1 month postvaccination, there were no consistent differences in antibody responses against HA between participants in the

Table 1. Baseline Characteristics of Trial Participants, Intention-to-Treat Population, N = 727

	Fluzone IIV4		Fluarix IIV4		ccIIV4		RIV4	
	n = 122		n = 120		n = 283		n = 202	
	n	%	n	%	n	%	n	%
Demographic characteristics								
Age (years), mean, (SD)	44	(11)	45	(11)	44	(11)	43	(12)
Age group, years								
18–44	57	(47)	55	(46)	135	(48)	102	(51)
45–64	65	(53)	65	(54)	148	(52)	100	(49)
Female	107	(88)	103	(86)	232	(82)	157	(78)
White	110	(90)	94	(78)	232	(82)	150	(74)
Hispanic	13	(11)	19	(16)	37	(13)	37	(18)
Site								
BSWH	73	(60)	75	(63)	147	(52)	151	(75)
KPNW	49	(40)	45	(37)	136	(48)	51	(25)
Baseline health characteristics[a]								
BMI, mean (SD)	28	(7)	30	(8)	29	(7)	29	(7)
Subjective health status, mean (SD)[b]	4	(1)	4	(1)	4	(1)	4	(1)
Diagnosed or treated for chronic medical condition during the past 12 months	16	(13)	22	(18)	29	(10)	31	(15)
Immunosuppressive condition	1	(1)	1	(1)	6	(2)	5	(2)
Smoker	3	(2)	1	(1)	11	(4)	13	(6)
Prior influenza vaccination receipt[c]								
Total vaccines received during the preceding 5 seasons, mean (SD)[c]	5	(1)	5	(1)	5	(1)	5	(1)
Received the 2017–2018 influenza vaccine	120	(98)	118	(98)	278	(98)	201	(99)

Abbreviations: BMI, body mass index; BSWH, Baylor Scott & White Health; ccIIV4, cell-culture based IIV4 represented by Flucelvax Quadrivalent™ by Seqirus; IIV4, quadrivalent inactivated influenza vaccine; KPNW, Kaiser Permanente Northwest; RIV4, recombinant IIV4 represented by Flublok by Sanofi Pasteur; SD, standard deviation.

[a]No participant was pregnant at enrollment.

[b]Original answer choice converted to numeric scale where 5 = excellent and 1 = poor.

[c]Based on report of vaccination by participant interview *or* electronic medical record extraction

ccIIV4 arm compared to the egg-based IIV4 arm for any vaccine reference viruses. In contrast, against A/H1N1 vaccine reference virus, participants in the RIV4 arm compared to the combined egg-based IIV4 arm had higher SCRs (29% vs 16%, $P < .01$, absolute difference 13%, $P < .01$), GMTs (99.7 vs 59.7, $P < .01$, GMT ratio 1.5, 95% confidence interval [CI] 1.2–1.9, $P < .01$), and MFRs (2.4 vs 1.8, $P < .01$) (Tables 2 and 3). Similarly, against the A/H3N2 vaccine reference virus, participants in the RIV4 arm had higher SCRs (55% vs 12%, $P < .01$, absolute difference 44%, $P < .01$), GMTs (339.2 vs 115.1, $P < .01$, GMT ratio 3.0, 95% CI: 2.4–3.7, $P < .01$), and MFRs (3.5 vs 1.2, $P < .01$) (Tables 2 and 3). Against the B/Victoria vaccine reference virus, participants in the RIV4 arm had higher GMTs and MFRs but not SCRs or GMT ratio. Against the B/Yamagata vaccine reference virus, participants in the RIV4 arm had higher SCRs (20% vs 10%, $P < .01$, absolute difference 10%, $P < .01$), GMTs (85.7 vs 65.7, $P = .01$), and MFRs (1.7 vs 1.3, $P < .01$) but not GMT ratio (1.1, 95% CI: .9–1.4, $P = .21$). Findings were generally similar when the analysis was limited to the one month per protocol population (Supplementary Table 3).

The small numbers of participants who received <5 influenza vaccines during the preceding 5 years precluded subgroup analyses to assess the interaction between number of prior influenza vaccinations during the preceding 5 years and vaccine type on seroconversion rates.

Antibody Responses at Six Months Postvaccination

At 6 months postvaccination, GMTs and GMT ratios did not differ between participants in the ccIIV4 arm compared to the combined egg-based IIV4 arm for any vaccine reference virus. Participants in the RIV4 arm had higher HI or MN GMTs compared to combined egg-based IIV4 recipients against the A/H3N2 and influenza B/Yamagata vaccine reference viruses (Figure 2). As a post hoc analysis, GMTs were analyzed by vaccine arm after excluding participants with reverse transcription polymerase chain reaction (RT-PCR)-confirmed ILI (5 in the combined egg-based IIV4 arm, 14 in the ccIIV4 arm, and 7 in the RIV4 arm, Supplementary Table 4) between the 1 and 6 month postvaccination visits. Findings were consistent with the per protocol analysis.

DISCUSSION

We evaluated the immunogenicity of quadrivalent cell-culture based and recombinant influenza vaccines compared to standard-dose egg-based vaccines among HCP aged 18–64 years using the same set of cell-grown influenza vaccine reference viruses for all vaccine types. Despite a history of frequent influenza vaccination among participants, egg-based IIV4, ccIIV4, and RIV4 all induced increases in

Table 2. Antibody Responses Prior to Vaccination and at One Month Postvaccination by Hemagglutination Inhibition or Microneutralization Against Cell-Grown Vaccine Reference Viruses[a] Among Recipients of Egg-Based, Cell-Based and Recombinant Influenza Vaccines, Intention-to-Treat Population,[b] N = 727

	Combined IIV4				ccIIV4					RIV4				
	n = 242				n = 283					n = 202				
	Day 0		1 month		Day 0		1 month		*P*-value[c]	Day 0		1 month		*P*-value[c]
Influenza A/H1N1, HI														
Seroconversion, no. (%, 95% CI)	…	…	39	(16, 11–21)	…	…	50	(18, 13-22)	.64	…	…	59	(29, 23–35)	<.01
Geometric mean titer (95% CI)	35.6	(30.0–42.2)	59.7	(50.1–71.2)	39.6	(34.0–46.1)	68.6	(59.2–79.3)	.23	47.2	(39.5–56.4)	99.7	(83.8–118.5)	<.01
Mean-fold rise in geometric titer (range)	…	…	1.8	(1.6–1.9)	…	…	1.8	(1.6–2.0)	.74	…	…	2.4	(2.0–2.6)	<.01
HI titer ≥1:40, no. (%, 95% CI)	140	(58, 52–64)	179	(74, 68–80)	175	(62, 56–68)	225	(80, 75–84)	.13	139	(69, 62–75)	172	(85, 80–90)	<.01
HI titer ≥1:80, no. (%, 95% CI)	103	(43, 36–49)	143	(59, 53–65)	121	(43, 37–49)	176	(62, 57–68)	.47	99	(49, 42–56)	146	(72, 66–78)	<.01
HI titer ≥1:160, no. (%, 95% CI)	54	(22, 17–28)	88	(36, 30–42)	60	(21, 16–26)	106	(37, 32–43)	.80	55	(27, 21–33)	108	(53, 47–60)	<.01
Influenza A/H3N2, MN														
Seroconversion, no. (%, 95% CI)	…	…	29	(12, 8–16)	…	…	48	(17, 13–21)	.11	…	…	112	(55, 49–62)	<.01
Geometric mean titer (range)	92.3	(78.0–109.2)	115.1	(96.4–137.4)	80.0	(68.8–93.0)	121.9	(103.5–143.6)	.64	95.6	(81.0–112.8)	339.2	(282.9–406.8)	<.01
Mean-fold rise in geometric titer (range)	…	…	1.2	(1.1–1.4)	…	…	1.5	(1.4–1.7)	.01	…	…	3.5	(2.9–4.3)	<.01
HI titer ≥1:40, no. (%, 95% CI)	203	(84, 79–89)	207	(86, 81–90)	232	(82, 78–86)	242	(86, 81–90)	.99	172	(85, 80–90)	192	(95, 92–98)	<.01
HI titer ≥1:80, no. (%, 95% CI)	166	(69, 63–74)	170	(70, 64–76)	182	(64, 59–70)	204	(72, 67–77)	.64	146	(72, 66–78)	187	(93, 89–96)	<.01
HI titer ≥1:160, no. (%, 95% CI)	105	(43, 37–50)	127	(52, 46–59)	103	(36, 31–42)	155	(55, 49–61)	.60	85	(42, 35–49)	172	(85, 80–90)	<.01
Influenza B/Victoria, HI														
Seroconversion, no. (%, 95% CI)	…	…	23	(10, 6–13)	…	…	23	(8, 5–11)	.58	…	…	29	(14, 10–19)	.11
Geometric mean titer (95% CI)	47.4	(41.3–54.3)	61.3	(53.6–70.1)	58.3	(52.0–65.4)	73.4	(65.8–82.0)	.04	48.0	(42.0–54.9)	75.0	(64.8–86.7)	.05
Mean-fold rise in geometric titer (range)	…	…	1.3	(1.2–1.5)	…	…	1.3	(1.2–1.4)	.49	…	…	1.7	(1.5–1.9)	<.01
HI titer ≥1:40, no. (%, 95% CI)	184	(76, 71–81)	208	(86, 82–90)	230	(81, 77–86)	253	(89, 86–93)	.23	157	(78, 72–83)	173	(86, 81–90)	.93
HI titer ≥1:80, no. (%, 95% CI)	114	(47, 41–53)	139	(57, 51–64)	160	(57, 51–62)	185	(65, 60–71)	.06	97	(48, 41–55)	135	(67, 60–73)	.04
HI titer ≥1:160, no. (%, 95% CI)	46	(19, 14–24)	65	(27, 21–32)	62	(22, 17–27)	92	(33, 27–38)	.16	33	(16, 11–21)	71	(35, 29–42)	.06
Influenza B/Yamagata, HI														
Seroconversion, no. (%, 95% CI)	…	…	23	(10, 6–13)	…	…	26	(9, 6–13)	.90	…	…	41	(20, 15–26)	<.01
Geometric mean titer (95% CI)	50.7	(43.8–58.8)	65.7	(56.5–76.3)	59.5	(52.2–67.8)	74.5	(65.8–84.4)	.20	53.9	(47.2–61.6)	85.7	(73.9–99.3)	.01
Mean-fold rise in geometric titer (range)	…	…	1.3	(1.2–1.5)	…	…	1.3	(1.2–1.4)	.59	…	…	1.7	(1.5–1.9)	<.01
HI titer ≥1:40, no. (%, 95% CI)	180	(74, 69–80)	194	(80, 75–85)	223	(79, 74–84)	236	(83, 79–88)	.34	151	(75, 69–81)	177	(88, 83–92)	.03
HI titer ≥1:80, no. (%, 95% CI)	127	(52, 46–59)	153	(63, 57–69)	162	(57, 51–63)	193	(68, 63–74)	.23	108	(53, 47–60)	137	(68, 61–74)	.31
HI titer ≥1:160, no. (%, 95% CI)	61	(25, 20–31)	83	(34, 28–40)	89	(31, 26–37)	102	(36, 30–42)	.68	40	(20, 14–25)	78	(39, 32–45)	.35

Abbreviations: ccIIV4, cell-culture based IIV4 represented by Flucelvax Quadrivalent™ by Seqirus; CI, confidence interval; HI, hemagglutination inhibition; IIV4, quadrivalent inactivated influenza vaccine represented by Fluzone by Sanofi Pasteur and Fluarix by GSK Biologics; RIV4, recombinant IIV4 represented by Flublok by Sanofi Pasteur.

[a]Cell-grown vaccine reference viruses: A/Michigan/45/2015; A/Singapore/INFIMH-16–0019/2016 SIAT1; B/Colorado/06/2017, ether treated; B/Phuket/3073/2013, ether treated.

[b]The intention-to-treat population comprised randomized participants meeting eligibility criteria regardless of vaccine receipt. To address missing data, a "worst-case" analysis approach was used in which a titer of 1:5 (ie, undetectable) was assigned for all missing data.

[c]*P*-value based on *t* test for postvaccination geometric mean titers and mean fold rises and χ^2 test for seroconversion rate and postvaccination titers ≥1:40, 1:80, 1:160 comparing either ccIIV4 or RIV4 recipients to combined egg-based IIV4 recipients.

Table 3. Geometric Mean Titer Ratios to Combined Egg-Based IIV4 Recipients at One Month Postvaccination by Hemagglutination Inhibition or Microneutralization Against Cell-Grown Vaccine Reference Viruses[a] by ccIIV4 and RIV4 Recipients, Intention-to-Treat Population,[b] N = 727

	ccIIV4			RIV4		
	n = 283			n = 202		
	GMT ratio[c]	95% CI	*P*-value	GMT ratio[c]	95% CI	*P*-value
Influenza A/H1N1, HI	1.0	(.8–1.2)	.99	1.5	(1.2–1.9)	<.01
Influenza A/H3N2, MN	0.9	(.8–1.2)	.62	3.0	(2.4–3.7)	<.01
Influenza B/Victoria, HI	1.0	(.9–1.2)	.62	1.1	(.9—1.3)	.53
Influenza B/Yamagata, MN	1.0	(.8–1.2)	.71	1.1	(.9–1.4)	.21

Abbreviations: CI, confidence interval; ccIIV4, cell-culture based IIV4 represented by Flucelvax Quadrivalent™ by Seqirus; HI, hemagglutination inhibition; IIV4, quadrivalent inactivated influenza vaccine represented by Fluzone by Sanofi Pasteur and Fluarix by GSK Biologics; RIV4, recombinant IIV4 represented by Flublok by Sanofi Pasteur.

[a]Cell-grown vaccine reference viruses: A/Michigan/45/2015; A/Singapore/INFIMH-16–0019/2016 SIAT1; B/Colorado/06/2017, ether treated; B/Phuket/3073/2013, ether treated.

[b]The intention-to-treat population comprised randomized participants meeting eligibility criteria regardless of vaccine receipt. To address missing data, a "worst-case" analysis approach was used in which a titer of 1:5 (ie, undetectable) was assigned for all missing data.

[c]Ratio of geometric mean titers at 1 month postvaccination among ccIIV4 recipients or RIV4 recipients compared to egg-based IIV4 recipients.

postvaccination antibody titers. RIV4 induced more robust antibody responses against HA than standard dose egg-based vaccines against the A/H1N1, A/H3N2, and B/Yamagata influenza vaccine strains at 1 month postvaccination, but response to the B/Victoria reference virus was similar. RIV4 recipients also had higher GMTs at 6 months postvaccination against 2 of the 4 vaccine strains (A/H3N2 and B/Yamagata), but GMT ratios comparing RIV4 recipients to egg-based vaccine recipients at 6 months were only significant for the A/H3N2 strain. In contrast, ccIIV4 induced similar responses against HA to all vaccine reference viruses at 1 and 6 months postvaccination compared to the egg-based vaccines. Our findings expand on those from previous studies that suggest that RIV4 may induce higher antibody responses against HA

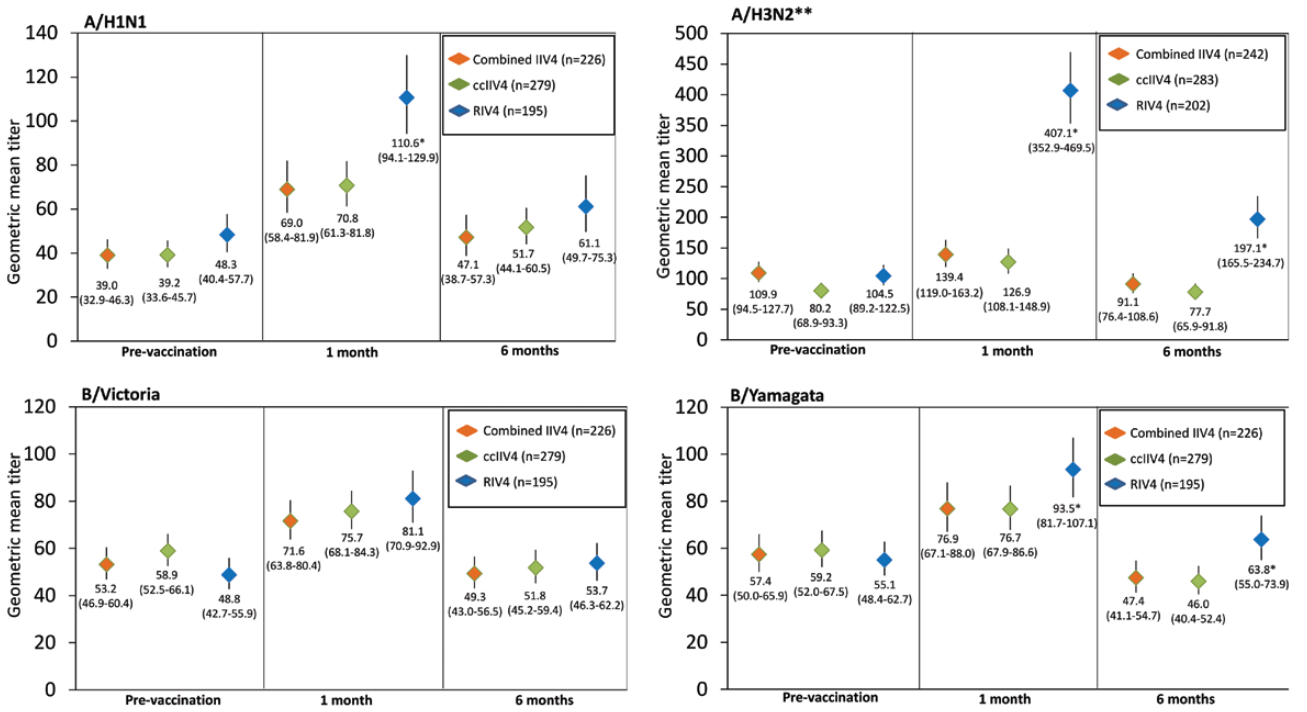

Figure 2. Geometric mean titers and 95% confidence intervals prior to vaccination and at 1 and 6 months postvaccination by hemagglutination inhibition or microneutralization against cell-grown vaccine reference viruses† among recipients of egg-based, cell-based and recombinant influenza vaccines, 1 and 6 month per protocol population‡, N = 700 at 1 month postvaccination. Abbreviations: ccIIV4, cell-culture based IIV4 represented by Flucelvax Quadrivalent™ by Seqirus; IIV4, quadrivalent inactivated influenza vaccine represented by Fluzone by Sanofi Pasteur and Fluarix by GSK Biologics; RIV4, recombinant IIV4 represented by Flublok by Sanofi Pasteur. †Cell-grown vaccine reference viruses: A/Michigan/45/2015; A/Singapore/INFIMH-16–0019/2016 SIAT1; B/Colorado/06/2017, ether treated; B/Phuket/3073/2013, ether treated. ‡The per protocol 1 and 6 month populations comprised randomized participants who received study vaccine and had serum samples drawn and tested within the protocol-specified acceptable time periods for each visit. *Indicates a statistically significant difference compared to the egg-based IIV4 recipients at the same time point based on a *P*-value < .05. **Microneutralization titers with different *y*-axis scale than other panes.

than IIV4 among adults in the general population [17–19] by demonstrating consistent effects among HCP with a history of frequent influenza vaccination. Primary outcomes from this trial focused on humoral immune responses to HA, which may not directly translate to differences in protection against or attenuation of laboratory-confirmed influenza. Although previous efficacy trials demonstrated that RIV is more efficacious than IIV against laboratory-confirmed influenza in older adults aged >50 years [23], large-scale efficacy trials are needed to assess whether RIV4 or 'ccIIV4 provide improved protection against laboratory-confirmed influenza outcomes in younger adult populations.

Our findings that RIV4 induced more robust antibody responses against HA to multiple cell-grown 2018–2019 vaccine reference viruses also expand upon findings from prior trials of RIV4 that largely assessed HI responses against BEVS-derived antigens or egg-derived antigens. The immunogenicity of RIV in adults was assessed in 5 prelicensure RCTs. In the 2 placebo-controlled trials conducted in different seasons using BEVS-derived antigens for all vaccine viruses [15] or egg- and cell-derived antigens [14], RIV (45 μg/antigen) induced higher antibody responses to the A/H1 and A/H3 vaccine viruses but not to the B viruses. In 3 active comparator trials from different seasons, RIV or IIV elicited higher antibody responses to the A/H1N1 vaccine viruses in 2 trials and A/H3N2 vaccine viruses in all 3 trials compared to the active comparator but similar responses to B viruses based on HI against BEVS-derived [13, 16] or egg-derived antigens [24]. An observational immunogenicity study conducted during the 2017–2018 influenza season that compared responses to egg-based IIV4, ccIIV4, RIV4, and high-dose IIV4 found similar antibody responses to RIV4 and high-dose IIV4 supporting the role of higher antigen in eliciting greater antibody responses [17]. RIV4 may also elicit improved immune responses beyond higher antibody titers such as more targeted immune responses to wild-type circulating viruses [17] and to parts of the viral HA that play a key role in infectivity [25].

The Center for Biologics Evaluation and Research criteria for noninferiority for influenza vaccine licensure are an upper bounds of the GMT ratio comparing the licensed product to the new product and absolute difference in SCR of 1.5 and 10%, respectively [26]. Similar criteria were used to determine superiority for immunogenicity outcomes in a phase III trial comparing high-dose to standard-dose influenza vaccine among persons ≥65 years [27]. In this trial, RIV4 recipients achieved these criteria when compared to egg-based IIV4 recipients for responses to the A/H3N2 vaccine reference virus (GMT lower bound 2.4 and absolute SCR 43.5%) but not the other vaccine reference viruses.

At least 2 possible limitations should be considered when interpreting study findings. First, this study was unable to assess the role of prior vaccination on humoral immune responses because most participants had received annual influenza vaccine during all 5 seasons preceding this trial. In addition, the study sample may have been subject to selection bias if HCP who agreed to participate were more accepting of influenza vaccine and thus more likely to be frequent vaccinees. Second, responses against the influenza neuraminidase were not assessed. Humoral immune responses to neuraminidase have been shown to reduce influenza illness severity [28, 29], and both cell-based and egg-based vaccine contain variable amounts of neuraminidase whereas RIV does not.

This trial was conducted among US HCP with a history of frequent vaccination that is likely representative of an increasing proportion of the US adult population given a decade-long recommendation for universal vaccination. Our findings that RIV4 elicited more robust humoral antibody responses against 3 of the 4 vaccine components compared to standard dose egg-based influenza vaccines add to emerging evidence [17–19] supporting a possible additional benefit from influenza vaccination with RIV or other vaccines with higher antigen content. Additional studies are needed to assess whether these findings remain consistent over multiple seasons with different vaccine strain compositions and across other markers of immune response and to assess vaccine efficacy and effectiveness against laboratory-confirmed outcomes.

Notes

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