Clinical Infectious Diseases

MAJOR ARTICLE

Immunogenicity of High-Dose Versus MF59-Adjuvanted Versus Standard Influenza Vaccine in Solid Organ Transplant Recipients: The Swiss/Spanish Trial in Solid Organ Transplantation on Prevention of Influenza (STOP-FLU Trial)

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Background. The immunogenicity of the standard influenza vaccine is reduced in solid-organ transplant (SOT) recipients, so new vaccination strategies are needed in this population.

Methods. Adult SOT recipients from 9 transplant clinics in Switzerland and Spain were enrolled if they were >3 months after transplantation. Patients were randomized (1:1:1) to a MF59-adjuvanted or a high-dose vaccine (intervention), or a standard vaccine (control), with stratification by organ and time from transplant. The primary outcome was vaccine response rate, defined as a ≥4-fold increase of hemagglutination-inhibition titers to at least 1 vaccine strain at 28 days postvaccination. Secondary outcomes included polymerase chain reaction–confirmed influenza and vaccine reactogenicity.

Results. A total of 619 patients were randomized, 616 received the assigned vaccines, and 598 had serum available for analysis of the primary endpoint (standard, n = 198; MF59-adjuvanted, n = 205; high-dose, n = 195 patients). Vaccine response rates were 42% (84/198) in the standard vaccine group, 60% (122/205) in the MF59-adjuvanted vaccine group, and 66% (129/195) in the high-dose vaccine group (difference in intervention vaccines vs standard vaccine, 0.20; 97.5% confidence interval [CI], .12–1); $P < .001$; difference in high-dose vs standard vaccine, 0.24 [95% CI, .16–1]; $P < .001$; difference in MF59-adjuvanted vs standard vaccine, 0.17 [97.5% CI, .08–1]; $P < .001$). Influenza occurred in 6% of the standard, 5% in the MF59-adjuvanted, and 7% in the high-dose vaccine groups. Vaccine-related adverse events occurred more frequently in the intervention vaccine groups, but most of the events were mild.

Conclusions. In SOT recipients, use of an MF59-adjuvanted or a high-dose influenza vaccine was safe and resulted in a higher vaccine response rate.

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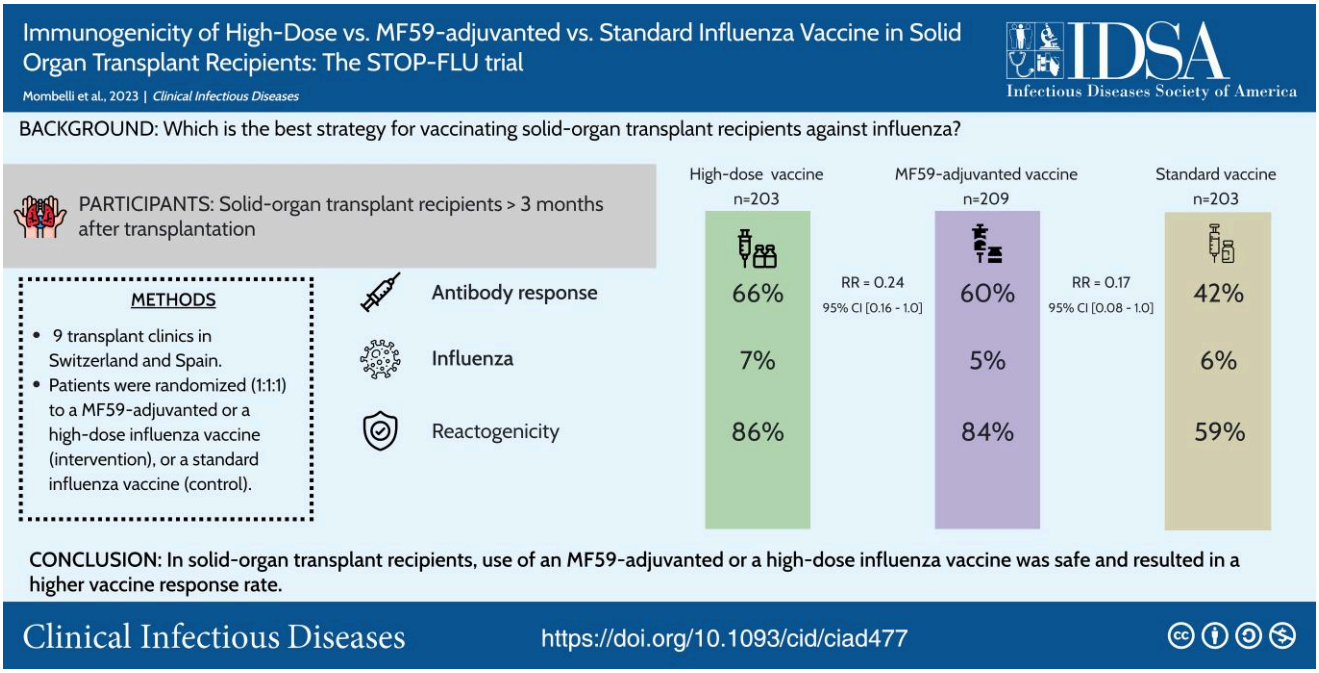
Graphical Abstract



Keywords. influenza; immunocompromised; vaccination; transplantation.

Seasonal influenza is associated with significant morbidity and mortality [1], particularly in immunocompromised patients [2]. Solid-organ transplant (SOT) recipients with influenza have higher rates of hospital admission, need for mechanical ventilation, and mortality compared with the general population [3–5]. Influenza has also been associated with reduced allograft survival in SOT recipients [5].

Annual immunization with the standard influenza vaccine is the mainstay of prevention and is recommended to all SOT recipients [6]. However, because of lifelong immunosuppression, the immunogenicity of influenza vaccine is reduced in SOT recipients [7–9]. Different strategies have been evaluated to increase vaccine responses in at-risk populations. Compared with the standard vaccine, adding the MF59 adjuvant [10] or increasing the dose of hemagglutinin antigens in the vaccine improved efficacy in the elderly [11, 12]. Preliminary data from small clinical trials in SOT recipients suggested improved immunogenicity with the use of MF59-adjuvanted and high-dose vaccines, although the efficacy in preventing influenza has not been evaluated [13–15].

We aimed to evaluate whether the MF59-adjuvanted and high-dose vaccines elicited better immunogenicity, were safe, and had better clinical efficacy compared with the standard influenza vaccine in SOT recipients.

METHODS

Study Design

The Swiss/Spanish Trial in Solid Organ Transplantation on Prevention of Influenza (STOP-FLU) was a double-blind, multicenter, randomized, superiority clinical trial of standard-dose nonadjuvanted influenza vaccine versus MF59-adjuvanted influenza vaccine versus high-dose influenza vaccine in SOT recipients. The protocol and statistical analysis plan are presented in the Supplementary Material.

Study participants were enrolled between October and December during 2 consecutive influenza seasons (2018–2019 and 2019–2020) at the outpatient transplant clinics of 6 participating centers during the first and second season (Basel, Bern, Geneva, Lausanne, Lugano, and Zurich in Switzerland) and at 3 additional centers during the second season (Chur and St. Gallen in Switzerland and Seville in Spain). The study protocol was approved by local ethics committees at each participating center (protocol numbers 2017-01922 in Switzerland and 2019-001974-27 in Spain) and was registered at Clinicaltrials.gov (NCT03699839).

Participants

We included adult (≥18 years old) SOT recipients who underwent transplantation at least 3 months before enrollment. Exclusion criteria were hypersensitivity to any component of the study vaccines, previous life-threatening reaction to influenza vaccine, ongoing therapy for rejection, current treatment with immunoglobulins or eculizumab, rituximab therapy within 6 months, ABO-incompatible transplantation, pregnancy/breastfeeding, and inability to comply with the study protocol. Patients who were vaccinated during the current vaccination campaign were also excluded. Patients enrolled during the first

year were excluded from participating again during the second year of recruitment. All participants provided written, informed consent.

Randomization and Masking

Eligible patients were centrally randomized in a 1:1:1 ratio to receive 1 intramuscular injection of the standard dose nonadjuvanted influenza vaccine (standard vaccine group), the MF59-adjuvanted influenza vaccine (MF59-adjuvanted vaccine group), or the high-dose influenza vaccine (high-dose vaccine group). A block randomization with varying block sizes was applied using the electronic Data Capture System SecuTrial. Randomization was stratified by type of organ (kidney vs others) and time after transplantation (up to 12 months vs more than 12 months). Because of delayed availability of the high-dose vaccine during the 2019–2020 season, block randomization was modified to allow initial allocation of participants to the standard and the MF59-adjuvanted vaccine only, with subsequent compensation when the high-dose vaccine became available. According to the allocated intervention, participants received one intramuscular injection in the deltoid of the nondominant arm of the vaccine by a nurse not involved in the trial. Because of slight differences in the suspension color between vaccines, participants were not allowed to look at the syringe during injection.

Procedures

Participants randomized in the standard vaccine group received VaxigripTetra (Sanofi-Pasteur MSD, France), those randomized in the MF59-adjuvated vaccine group received Fluad (PaxVax Berna GmbH, Switzerland), and those randomized in the high-dose vaccine group received Fluzone-HD (Sanofi-Pasteur, France). All are commercially available, inactivated, split-virion influenza vaccines. VaxigripTetra is a quadrivalent nonadjuvanted influenza vaccine containing 15 μg of hemagglutinin antigen per strain. Fluad is a trivalent influenza vaccine containing 15 μg of hemagglutinin antigen per strain and the adjuvant MF59. Fluzone-HD is a trivalent nonadjuvanted influenza vaccine containing 60 μg of hemagglutinin antigen per strain. The influenza strains contained in the vaccines are summarized in Supplementary Table 1. The quadrivalent vaccine (VaxigripTetra) contained an additional B strain (Yamagata lineage) for both seasons.

Serum samples were collected at baseline and days 28 and 180 after vaccination for immunogenicity and anti–human leukocyte antigen (HLA) antibodies analysis. Study participants were asked to record and grade solicited local and systemic adverse events (detailed in Supplementary Table 2) as well as body temperature on a diary card within 7 days after vaccination.

During the influenza season, patients were instructed to refer to their transplant center in case of symptoms suggestive of influenza-like illness (cough, fever, and/or sudden onset of illness) to perform an influenza-specific polymerase chain reaction (PCR) test in the nasopharyngeal swab. In addition, each study participant was instructed by a study nurse to collect 5 nasopharyngeal swabs (flocked swabs with viral transport media) at weeks 2, 4, 6, 8, and 10 after the onset of the influenza season and to fill in a questionnaire asking for symptoms of influenza at the time of each swab. Patients performed the swab themselves and couriered it to the trial central laboratory (Diagnostic Laboratory at the University Hospital Basel, Switzerland). Patients were followed up to 180 days after vaccination.

Immunogenicity was assessed by the measurement of hemagglutinin-inhibition (HAI) titers using the hemagglutinin-inhibition assay performed in the same batch at the Department of Biomedicine of the University of Basel, following the standardized World Health Organization methods [16–18]. Briefly, serum was incubated with standardized amounts of influenza antigens followed by addition of a suspension of 0.5% red blood cells. Geometric mean titers (GMTs) were determined by doubling dilutions of antibody starting from an initial dilution of 1:8 to a final dilution of 1:1024. Samples with a negative result were assigned a titer of 1:4. Internal positive (same highly positive serum) and negative controls were used.

Influenza-specific PCR tests were performed as part of routine clinical practice at the treating center in case of symptomatic infection. Influenza-specific PCR on surveillance nasopharyngeal swabs were performed at the diagnostic laboratory at the University Hospital Basel according to established protocols with RNA extraction on an Abbott robotic system (m2000sp, Switzerland) followed by PCR against Influenza A and B with a kit from Altona Diagnostics (Hamburg, Germany) on an ABI7500 light cycler (ThermoFisher, Switzerland). Anti-HLA antibodies were measured by solid-phase assay on beads (LABScreen mixed; OneLambda, ThermoFischer, Switzerland) and the titer was determined by the mean fluorescence index of each specific bead, according to standard procedures.

Outcomes

The primary outcome of the study was the vaccine response rate at day 28, defined as the proportion of patients exhibiting seroconversion for at least 1 viral strain (A/H1N1, A/H3N2, or B) contained in the trivalent vaccines at day 28 after vaccination. Seroconversion was defined as an at least 4-fold increase of HAI titer from baseline. Secondary immunogenicity outcomes were GMTs of HAI titers, seroprotection rates, seroconversion rates, and seroconversion factors for each vaccine strain at days 28 and 180. Seroprotection was defined as an HAI titer of 1:40 or greater, and seroconversion factor was defined as the fold increase in anti-HAI titers for each viral strain before and after vaccination, according to the standard definition of the European Agency for the Evaluation of Medical Products. A seroconversion factor of 2.5 or greater is required [19]. The secondary clinical outcome was the proportion of participants with clinical or subclinical influenza confirmed by PCR by day 180. Safety outcomes included vaccine reactogenicity,

defined as the proportion of participants with local or systemic solicited adverse events by day 7, and the proportion of participants with de novo anti-HLA antibodies, biopsy-proven acute rejection, and death by day 180 after vaccination.

Statistical Analysis

We considered that the lowest seroconversion rate would be of 46% with the standard vaccine, that the mid-seroconversion rate would be of 59% with the MF59-adjuvanted vaccine, and the highest seroconversion rate would be of 70% with the high-dose vaccine [13, 20]. Thus, considering a dropout rate of 10%, assuming a power of 80% and a family-wise error rate of 5%, we planned to enroll 780 patients (260 per group) to test for superiority of the intervention vaccines over the control vaccine, using Bonferroni-Holm adjustment to define significance levels per test. Because we did not reach the planned number of patients after 2 years of recruitment, we considered extending the inclusion of patients for a third year. Unfortunately, the advent of the coronavirus disease 2019 pandemic precluded the extension of the trial.

Primary analysis was performed in participants who received the allocated vaccine and for whom HAI titer measurement at baseline and day 28 was available and thus were evaluable for the primary outcome (per protocol). To compare the 2 intervention vaccines and the standard vaccine, a hierarchical testing strategy with Bonferroni-Holm adjustment was performed. We defined 2 levels of hypotheses, treated the first as a "gatekeeper" and tested the second level of hypotheses only if 1 or more gatekeeper hypotheses were rejected. All hypotheses defined later were formulated as superiority of the first named over the second measured as absolute differences in vaccine response rates at day 28 (1-sided tests). The first level of hypotheses comprised the comparison of both interventional vaccines together against standard vaccine (H1, significance level $\alpha = 2.5\%$) and high-dose alone against standard vaccine (H2, $\alpha = 5\%$). Comparisons of MF59-adjuvanted against standard (H3, $\alpha = 2.5\%$) and high-dose versus MF59-adjuvanted vaccine (H4, $\alpha = 5\%$) were defined on the second level of hypotheses. We used a linear mixed model to analyze predictors of vaccine response with transplantation center as a random intercept. Secondary analysis included the comparison of the proportion of participants with clinical or subclinical PCR-confirmed influenza at day 180 and of additional immunogenicity parameters among the 3 groups. Safety analysis was

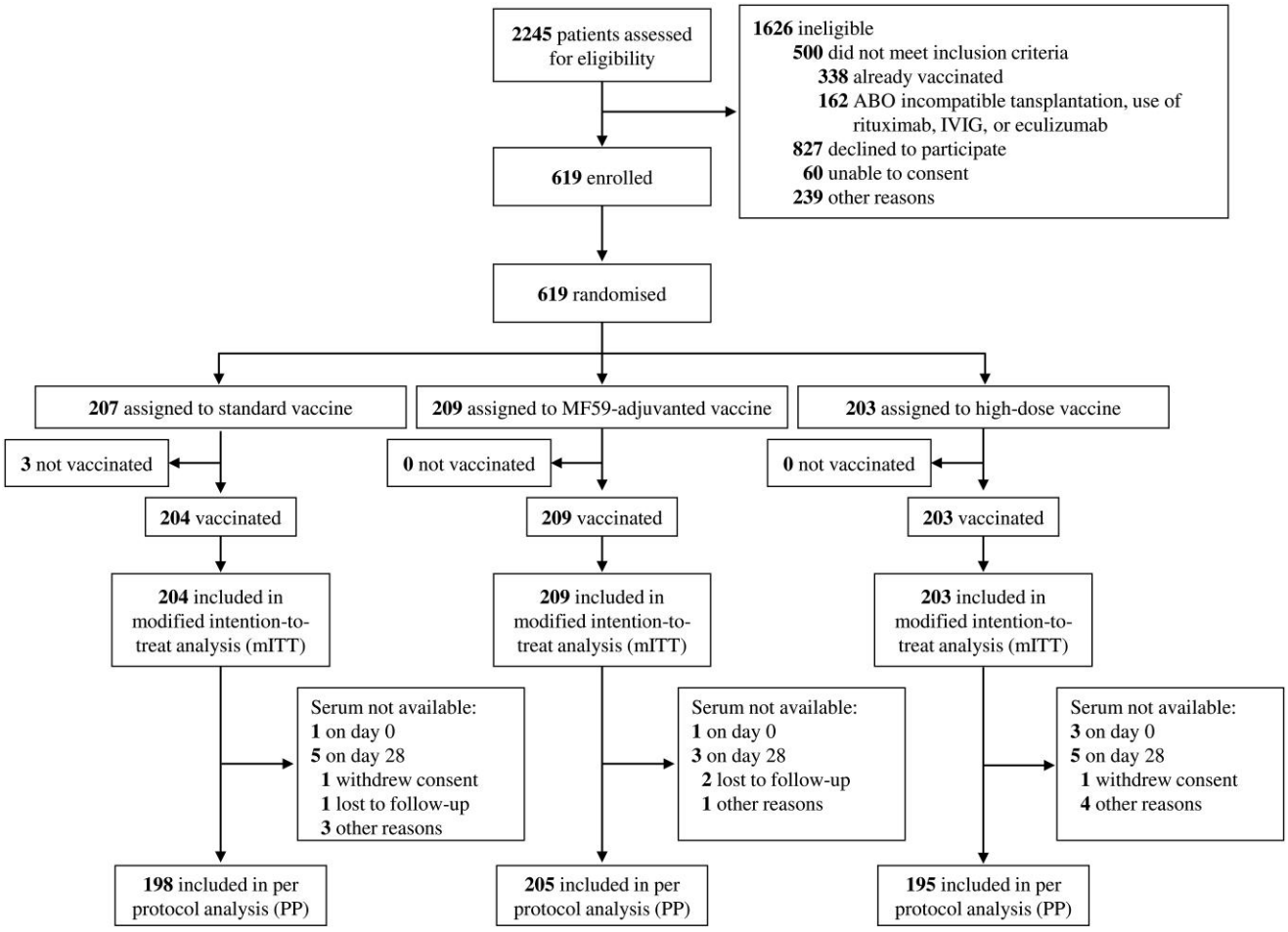


Figure 1. Trial profile.

Table 1. Baseline Characteristics of the Participants Included in the Modified Intention-to-Treat Population

	Standard Vaccine (n = 204)	MF59-Adjuvanted Vaccine (n = 209)	High-Dose Vaccine (n = 203)
Age, median (IQR)	58 (49, 65)	57 (45, 64)	56 (47, 66)
Sex (male), n (%)	150 (74)	148 (71)	139 (69)
Months after transplantation, median (IQR)	30 (11, 108)	49 (11, 109)	57 (12, 120)
Less than 1 year after transplantation, n (%)	57 (28)	56 (27)	52 (26)
Transplanted organ			
Kidney	140 (69)	140 (67)	136 (67)
Liver	44 (22)	43 (21)	29 (14)
Heart	10 (5)	10 (5)	16 (8)
Lung	6 (3)	6 (3)	13 (6)
Pancreas	1 (0.5)	2 (1)	4 (2)
Combined[a]	3 (2)	8 (4)	5 (3)
Previous transplantation	21 (10)	26 (13)	20 (10)
Induction immunosuppression[b], n (%)			
ATG	25 (13)	28 (14)	27 (14)
Basiliximab	116 (59)	96 (47)	90 (47)
Other	34 (17)	36 (17)	22 (11)
Maintenance immunosuppression, n (%)			
Tacrolimus	145 (71)	148 (71)	141 (70)
Cyclosporin	37 (18)	40 (19)	44 (22)
Mycophenolate	165 (81)	161 (77)	150 (74)
Azathioprine	7 (3)	8 (4)	21 (10)
mTOR inhibitor	20 (10)	22 (11)	14 (7)
Prednisone	119 (58)	136 (65)	120 (59)
Other	5 (3)	8 (4)	4 (2)
Influenza vaccine in the previous season[c], n (%)	169 (83)	176 (84)	166 (82)
Previous influenza vaccine[d], n (%)	178 (87)	190 (91)	177 (88)

Abbreviations: ATG, antithymocyte globulins; IQR, interquartile range; mTOR, mechanistic target of rapamycin.

[a]Including 8 kidney-pancreas, 4 kidney-liver, 3 kidney-heart, and 1 kidney-lung transplant recipients.

[b]Induction immunosuppression was missing for 77 participants.

[c]Influenza vaccine during the previous season was unknown for 13 participants.

[d]Previous influenza vaccine was unknown for 14 participants.

Table 2. Primary Outcome for Patients Receiving the High-Dose, MF59-Adjuvanted and Standard Influenza Vaccines in the Per-Protocol Population

	Vaccine Response Rate	Risk Difference	*P* Value
High-dose and MF59-adjuvanted versus standard vaccine[a]	63% (251/400) versus 42% (84/198)	0.20 (97.5% CI, .12–1)	<.001
High-dose versus standard vaccine[a]	66% (129/195) versus 42% (84/198)	0.24 (95% CI, .16–1)	<.001
MF59-adjuvanted versus standard vaccine[b]	60% (122/205) versus 42% (84/198)	0.17 (97.5% CI, .08–1)	<.001
High-dose versus MF59-adjuvanted vaccine[b]	66% (129/195) versus 60% (122/205)	0.07 (95% CI, −.01 to 1)	.085

Abbreviation: CI, confidence interval.

[a]First-level hypothesis.

[b]Second-level hypothesis. A 2-level hierarchical test procedure for differences in response rates to the vaccine at day 28 postvaccination was applied. The tests for the hypotheses at the first level served as "gatekeepers." The second-level hypotheses were only tested if 2 or more gatekeeper null hypotheses were rejected.

performed in all participants who received the allocated treatment (modified intention-to-treat [mITT]). Analysis was performed using R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study Population

Between October 2018 and December 2019, 2245 SOT recipients were assessed for eligibility, 619 were randomized, and 616 received the standard (n = 204), the MF59-adjuvanted (n = 209), or the high-dose (n = 203) vaccines and were included in the mITT population (Figure 1). Serum for HAI titers analysis was obtained in 598 participants: standard (n = 198), MF59-adjuvanted (n = 205), and high-dose (n = 195) groups (per protocol population, Figure 1). Characteristics of the participants are shown in Table 1. Most of the participants were male with a median age of 58 years. Sixty-eight percent of participants were kidney transplant recipients and median time after transplantation was 42 months. Of the 616 participants, 434 (71%) were on tacrolimus, 476 (77%) on mycophenolate, and

Table 3. Immunogenicity of the Influenza Vaccine in the Per-Protocol Population

	Standard (n = 198)	MF59-Adjuvanted (n = 205)	High-Dose (n = 195)
Anti-influenza antibody titers, GMTs (95% CI)			
H1N1			
Baseline	30.58 (25.7–36.38)	32.22 (27.11–38.28)	24.95 (20.84–29.88)
Day 28	53.91 (45.45–63.94)	76.56 (64.38–91.04)	85.96 (70.97–104.13)
Day 180[a]	39.78 (33.37–47.43)	56.81 (47.63–67.77)	50.36 (42.02–60.37)
H3N2			
Baseline	13.01 (11.01–15.39)	12.04 (10.43–13.91)	11.17 (9.62–12.98)
Day 28	32.34 (26.48–39.5)	50.85 (41.68–62.04)	56.11 (44.61–70.58)
Day 180[a]	25.09 (20.7–30.41)	29.13 (24.43–34.74)	32.72 (26.51–40.37)
B			
Baseline	11.8 (10.13–13.74)	12.29 (10.56–14.31)	10.48 (9.01–12.19)
Day 28	18.53 (15.57–22.06)	24.17 (20.41–28.62)	25.95 (21.92–30.71)
Day 180[a]	14.19 (12.1–16.65)	17.14 (14.61–20.1)	18.14 (15.53–21.18)
Seroconversion rates at day 28, n (%)			
H1N1	34 (17)	55 (27)	91 (47)
H3N2	69 (35)	106 (52)	111 (57)
B	25 (13)	52 (25)	63 (32)
Seroconversion rates at day 180, n (%)			
H1N1	21 (11)	36 (19)	53 (28)
H3N2	51 (27)	68 (35)	87 (46)
B	7 (4)	23 (12)	37 (20)
Seroconversion factors at day 28, GMTs (95% CI)			
H1N1	1.76 (1.55–2)	2.38 (2.08–2.72)	3.45 (2.89–4.11)
H3N2	2.48 (2.14–2.88)	4.22 (3.51–5.07)	5.02 (4.09–6.16)
B	1.57 (1.43–1.72)	1.97 (1.75–2.21)	2.48 (2.14–2.86)
Seroconversion factors at day 180, GMTs (95% CI)			
H1N1	1.32 (1.19–1.46)	1.65 (1.45–1.87)	2.03 (1.75–2.35)
H3N2	1.96 (1.71–2.26)	2.39 (2.06–2.77)	2.85 (2.41–3.37)
B	1.21 (1.13–1.3)	1.36 (1.25–1.49)	1.69 (1.52–1.89)
Seroprotection rates, n (%)			
H1N1			
Baseline	126 (64)	129 (63)	100 (51)
Day 28	159 (80)	174 (85)	162 (83)
Day 180[a]	140 (73)	153 (80)	136 (72)
H3N2			
Baseline	60 (30)	55 (27)	52 (27)
Day 28	122 (62)	146 (71)	139 (71)
Day 180[a]	99 (52)	116 (60)	107 (57)
B			
Baseline	49 (25)	63 (31)	48 (25)
Day 28	80 (40)	109 (53)	110 (56)
Day 180[a]	61 (32)	83 (43)	83 (44)

Abbreviations: CI, confidence interval; GMT, geometric mean titer.

[a]Serum samples were missing at day 180 for 27 of the participants (7 in the standard, 13 in the adjuvanted, and 7 in the high-dose vaccine group, respectively).

375 (61%) on steroids. Overall, 511/616 (83%) participants received influenza vaccination during the previous season.

Primary Outcome

Vaccine response at 28 days occurred in 84 of 198 participants (42%) in the standard vaccine group, 122 of 205 (60%) in the MF59-adjuvanted vaccine group, and 129 of 195 (66%) in the high-dose vaccine group. Difference in vaccine response rate was 0.20 (97.5% confidence interval [CI], .12–1; $P < .001$) in the intervention vaccine groups versus standard vaccine group, 0.24 (95% CI, .16–1; $P < .001$) in the high-dose versus standard vaccine group, and 0.17 (97.5% CI, .08–1; $P < .001$) in the MF59-adjuvanted versus standard vaccine group (Table 2). No difference was observed between the high-dose and MF59-adjuvanted vaccines (difference 0.07; 95% CI, −.01–1; $P = .085$).

Secondary Outcomes

Other immunogenicity parameters are illustrated in Table 3 and in Supplementary Figure 1. GMT titers and seroconversion rates for each viral strain were generally higher in the intervention vaccine groups at day 28 (seroconversion rates ranging from 25% to 57% depending on the vaccine strain) compared with the standard

Table 4. Episodes of Microbiologically Confirmed Influenza Included in the Per-Protocol Population

	Standard Vaccine (n = 198)	MF59-Adjuvanted Vaccine (n = 205)	High-Dose Vaccine (n = 195)
Patients with influenza, n (%)	11 (6)	11 (5)	13 (7)
Median days from vaccination to influenza (IQR)	91 (89, 106)	70 (66, 89)	96 (68, 103)
Viral strain			
A H1N1	5 (3)	5 (2)	4 (2)
A nonspecified	4 (2)	5 (2)	5 (3)
B	2 (1)	1 (0.5)	4 (2)
Influenza season			
2018/2019	5 (3)	6 (3)	5 (3)
2019/2020	6 (3)	5 (2)	8 (4)
Symptomatic influenza, (%)	8 (4)	8 (4)	10 (5)
Diagnosed by surveillance PCR[a], n (%)	10 (5)	7 (3)	9 (5)
Clinical outcomes			
Viral pneumonia, n (%)	0 (0)	0 (0)	0 (0)
Bacterial pneumonia, n (%)	0 (0)	1 (0.5)	1 (0.5)
Hospital admission, n (%)	0 (0)	1 (0.5)	1 (0.5)
ICU admission, n (%)	0 (0)	0 (0)	0 (0)

Abbreviations: ICU, intensive care unit; IQR, interquartile range; PCR, polymerase chain reaction.

[a]Influenza was diagnosed both during routine practice and surveillance PCR in 3 participants.

Table 5. Reactogenicity and Safety of Influenza Vaccines in the Modified Intention-to-Treat Population

Events, n (%)	Standard Vaccine (n = 204)	MF59-Adjuvanted Vaccine (n = 209)	High-Dose Vaccine (n = 203)
Any solicited adverse event, n (%)	121 (59)	177 (84)	175 (86)
Local solicited adverse event, n (%)			
Pain	45 (22)	106 (51)	84 (41)
Redness	16 (8)	26 (12)	23 (11)
Swelling	22 (11)	36 (17)	29 (14)
Systemic solicited adverse event, n (%)			
Arthralgia	19 (9)	26 (12)	21 (10)
Fatigue	55 (27)	60 (29)	66 (33)
Fever	5 (2)	12 (6)	15 (7)
Headache	31 (15)	37 (18)	50 (25)
Myalgia	29 (14)	33 (16)	38 (19)
Nausea	8 (4)	12 (6)	22 (11)
Vomiting	2 (1)	2 (1)	7 (3)
Serious adverse events, n (%)	48 (24)	28 (13)	34 (17)
Vaccine-related serious adverse events	0 (0)	0 (0)	1 (0.5)
Biopsy-proven acute rejection, n (%)	5 (2)	1 (0.5)	3 (1)
De novo anti-HLA antibodies, n (%)			
Class I	4 (2)	7 (3)	5 (2)
Class II	1 (0.5)	4 (2)	1 (0.5)
Class I and Class II	3 (1)	3 (1)	2 (1)
Death	0 (0)	2 (1)	0 (0)

Adverse events were evaluated in the 616 participants included in the modified intention-to-treat population.

Abbreviation: HLA, human leukocyte antigen.

vaccine group (13% to 35%). Seroprotection rates at day 28 were also higher in the intervention vaccine groups. Prespecified predictors of vaccine response are shown in Supplementary Table 3.

Microbiologically confirmed influenza occurred in 35/598 (6%) participants, without differences between groups (Table 4). Of the 35 episodes of influenza, 23 (66%) were diagnosed only through surveillance testing. All 9 episodes diagnosed during routine clinical practice were treated with oseltamivir. Among those, 2 participants had bacterial pneumonia and were admitted to the hospital (1 in the MF59-adjuvanted vaccine group and 1 in the high-dose vaccine group). None of the participants was admitted to the intensive care unit or died because of influenza.

Safety

All 3 vaccines were safe and well tolerated. During the first 7 days after vaccination, solicited adverse events occurred in 121/204 participants (59%) in the standard vaccine group, 177/209 (84%) in the MF59-adjuvanted vaccine group, and 175/203 (86%) in the high-dose vaccine group (Table 5). Most of the solicited adverse events were mild and self-limited (Supplementary Table 4). Serious adverse events occurred in 48/204 (24%) participants in the standard, 28/209 (13%) in the MF59-adjuvanted, and 34/203 (17%) in the high-dose vaccine groups. Only 1 of those serious adverse events was correlated with vaccination (panniculitis 3 days after high-dose vaccination). Rates of de novo anti-HLA antibodies and biopsy proven acute rejection were low among all vaccine groups (Table 5). Two patients died during follow-up of a cause not related with vaccination or influenza (1 suicide and 1 sudden death).

DISCUSSION

In this randomized clinical trial, we found that SOT recipients receiving the MF59-adjuvanted and high-dose vaccines had a higher humoral response than patients receiving the standard influenza vaccine. All vaccines were well-tolerated, and we did not observe differences in anti-HLA antibodies or acute rejection after vaccination between groups. The overall incidence of clinical and subclinical influenza was comparable between groups.

Our results are in accordance with previous studies demonstrating improved humoral responses to the high-dose and MF59-adjuvanted vaccines in the elderly [11, 21, 22]. However, evidence for a benefit of these alternative vaccination strategies specifically in the transplant population is weaker,

relying only on small clinical trials. In 1 trial enrolling 166 SOT recipients, vaccine responses were significantly higher in the high-dose (79%) compared with the standard-dose vaccine group (53%) [14]. In additional studies, a trend toward increased immunogenicity was observed with a double-dose or a high-dose vaccine [20, 23]. The immunogenicity of the MF59-adjuvanted was also compared with the standard influenza vaccine in 2 clinical trials, with no significant differences despite a trend toward increased immunogenicity [13, 24]. Overall, our trial provides the most robust evidence so far on the improved immunogenicity of the high-dose and MF59-adjuvanted vaccines in SOT recipients.

We asked study participants to systematically perform nasopharyngeal swabs during the influenza seasons. We observed a higher incidence of breakthrough influenza (6%), with most infections detected through systematic screening. This is an important difference with previous trials performed in the transplant population in which the low incidence of influenza (1.0%–1.8%) precluded any analysis of the clinical efficacy [13, 14, 25]. Despite higher antibody titers observed in the intervention vaccines arms, we did not observe differences in the incidence of influenza. This can be explained by the fact that a large proportion (83%) of the patients included in the trial have received previous influenza vaccination, so that some of these patients may have some degree of baseline protection against disease, in particular for severe complications of influenza, but not for mild upper respiratory tract infection. Also, although this is 1 of the largest trials performed in transplant recipients, a larger sample size might have been necessary to detect a small effect of the intervention on breakthrough influenza, as observed with the high-dose vaccine in the elderly [11]. Finally, it is unlikely that the lack of improved efficacy of intervention vaccines is explained by the additional protection against B strain (Yamagata lineage) that was included only in the quadrivalent standard vaccine because influenza B/Yamagata strains were not detected in Switzerland and Spain during the study period. This is in line with the observed decrease in the incidence of influenza B/Yamagata in the context of the coronavirus disease 2019 pandemic [26]. Related to that, both MF59-adjuvanted and high-dose quadrivalent influenza vaccines are now available and approved in people aged 65 years or older [27].

Overall, the 3 vaccines were safe and well tolerated. As previously observed, vaccine reactogenicity, particularly pain at injection site, was more common with the MF9-adjuvanted and the high-dose vaccines, and most of the adverse events were mild and spontaneously resolving [13, 14, 28]. We did not observe any significant allograft-related adverse outcomes with the use of the MF59-adjuvanted or the high-dose vaccines, confirming the safety of influenza vaccination in this population [29, 30].

We identified several known variables associated with higher likelihood of vaccine response in our population. These variables include liver and pancreas transplantation, vaccination occurring ≥12 months posttransplant, an immunosuppressive regimen without mycophenolate, and absence of vaccination in the previous season. Regarding the latter variable, 1 possible explanation is that higher baseline antibody titers might hinder the achievement of a 4-fold increase in antibody titers after vaccination [31]. Of note, the control group had a shorter median time from transplantation to enrollment. Nevertheless, the percentage of patients vaccinated during the first year after transplantation was comparable between groups because randomization was stratified based on the time posttransplant.

Our trial has several limitations. First, the primary outcome of the trial was vaccine immune response and not clinical efficacy, which would have required a much larger sample size. Second, we did not measure influenza specific cell-mediated immunity for assessing the primary outcome. Although cell-mediated immunity may be a more accurate marker for protection against severe disease, measurement of HAI titers is widely used as a marker for vaccine response in most clinical trials [32]. Finally, kidney transplant recipients after transplantation were overrepresented in the trial, so that the results of the study may not be extrapolated to all transplant populations.

In conclusion, in SOT recipients the use of the MF59-adjuvanted and the high-dose influenza vaccines resulted in a higher vaccine response compared with the standard vaccine without safety concerns. Despite uncertainty regarding clinical outcomes, our results provide evidence suggesting that these vaccines are preferable to the standard vaccine for preventing influenza in SOT recipients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. M. M., M. P., C. B., N. J. M., A. E., C. v. D., and O. M. conceived and designed the study. D. N., U. H. D., J. G. C., D. G., S. D., G. S., A. S., C. G., R. M. V., L. M., M. S., L. W., C. H., A. M., M. D., J. D. A., J. S., T. F. M., M. C., I. B., J. V., E. C., and C. v. D. were responsible for the acquisition of data. M. M., S. S., M. K., and O. M. performed the analyses and interpreted the results in collaboration with all other authors. M. M. and O. M. wrote the first draft of the report. All authors critically revised the report for important intellectual content and approved the final version.

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Data sharing statement. Deidentified, individual participant data that underlie this article, along with a data dictionary describing variables in the dataset, are available to researchers whose proposed purpose of use is approved by the Scientific Committee of the Swiss Transplant Cohort Study. Related documents such as the study protocol and informed consent form will be made available on request. To request the dataset, please send a signed data request form to oriol.manuel@chuv.ch.

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