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ORIGINAL ARTICLE

A pilot randomized trial of adjuvanted influenza vaccine in adult allogeneic hematopoietic stem cell transplant recipients

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The annual influenza vaccine is recommended for hematopoietic stem cell transplant (HSCT) patients although studies have shown suboptimal immunogenicity. Influenza vaccine containing an oil-in-water emulsion adjuvant (MF59) may lead to greater immunogenicity in HSCT recipients. We randomized adult allogeneic HSCT patients to receive the 2015–2016 influenza vaccine with or without MF59 adjuvant. Preimmunization and 4-week post-immunization sera underwent strain-specific hemagglutination inhibition assay. We randomized 73 patients and 67 (35 adjuvanted; 32 non-adjuvanted) had paired samples available at follow-up. Median age was 54 years (range 22–74) and time from transplant was 380 days (range 85–8107). Concurrent graft-versus-host disease was seen in 42/73 (57.5%). Geometric mean titers increased significantly after vaccination in both groups. Seroconversion to at least one of three influenza antigens was present in 62.9% vs 53.1% in adjuvanted vs non-adjuvanted vaccine ($P = 0.42$). Factors associated with lower seroconversion rates were use of calcineurin inhibitors ($P < 0.001$) and shorter duration from transplantation ($P = 0.001$). Seroconversion rates were greater in patients who got previous year influenza vaccination (82.6% vs 45.5%, $P = 0.03$). Adjuvanted vaccine demonstrated similar immunogenicity to non-adjuvanted vaccine in the HSCT population and may be an option for some patients.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) offers a curative strategy for patients with hematological malignancy. Influenza is an important cause of morbidity in this population and can lead to lower respiratory complications and death.[1,2] HSCT patients infected with influenza have been shown to have high influenza viral loads and may shed virus for prolonged periods.[3] Annual influenza vaccination is currently considered the main strategy to prevent influenza after HSCT. The current inactivated vaccine contains contains 15 μg antigen from each of two circulating subtypes of influenza A and 15 μg of an influenza B subtype (www.who.int). However, vaccine appears to be less effective in immunosuppressed persons such as HSCT recipients, particularly during the first several months after transplantation, or in patients with GVHD receiving immunosuppressive treatments.[4–9]

One potential method of increasing immunogenicity is by using an adjuvanted influenza vaccine. Two adjuvants have been used in commercially available seasonal influenza vaccines: AS03 and MF59. AS03 was used in the monovalent pandemic A/H1N1 vaccine in Canada and Europe but not further developed for seasonal vaccines. MF59 adjuvant has been authorized for use in seasonal influenza vaccine in Canada and Europe for persons $\geqslant 65$ years of age and recently approved for use by the United States Food and Drug Administration. MF59 is an oil-in water emulsion that is packaged as small microvesicles.[10] The complete mechanism of action of MF59 is not well understood but requires activation of the innate immune system;[11] the adjuvant exerts a local inflammatory response increasing the influx of neutrophils and macrophages to the injection site. These cells secrete chemokines, thereby recruiting monocytes that differentiate into dendritic cells. MF59 further promotes uptake of the antigen into newly recruited dendritic cells. MF59-adjuvanted influenza vaccine may have the potential to increase immunogenicity in immunosuppressive conditions but has not been previously studied in the HSCT population.

Therefore, we conducted a randomized trial comparing a novel strategy of adjuvanted influenza vaccination vs standard non-adjuvanted vaccination. We hypothesized that due to the adjuvant's mechanism of action, the adjuvanted vaccine would result in improved immunogenicity and equivalent safety in this very susceptible population.

MATERIALS AND METHODS

Patient population and study design

The study was conducted at a tertiary care cancer center after receiving institutional research ethics board approval. All patients provided written informed consent. Adult allogeneic HSCT recipients attending outpatient clinics were randomized to receive adjuvanted or non-adjuvanted influenza vaccine during the 2015–2016 season. Patients were included if they were at least 12 weeks after transplantation and had not previously received the 2015–2016 influenza vaccine. We excluded patients who were receiving regular intravenous immunoglobulin (IVIG) as well as those who had relapse of their underlying disease. At enrollment, patients received either adjuvanted or non-adjuvanted seasonal influenza vaccine in a 1:1 ratio. Randomization was done using a computer-generated schedule in blocks of four to ensure equal numbers in each group. Both vaccines contained 15 μg each of the same three influenza antigens in 0.5 mL volume: A/California/7/2009 (H1N1)pdm09; A/Switzerland/9715293/2013 (H3N2); B/Phuket/3073/2013 (Yamagata lineage).

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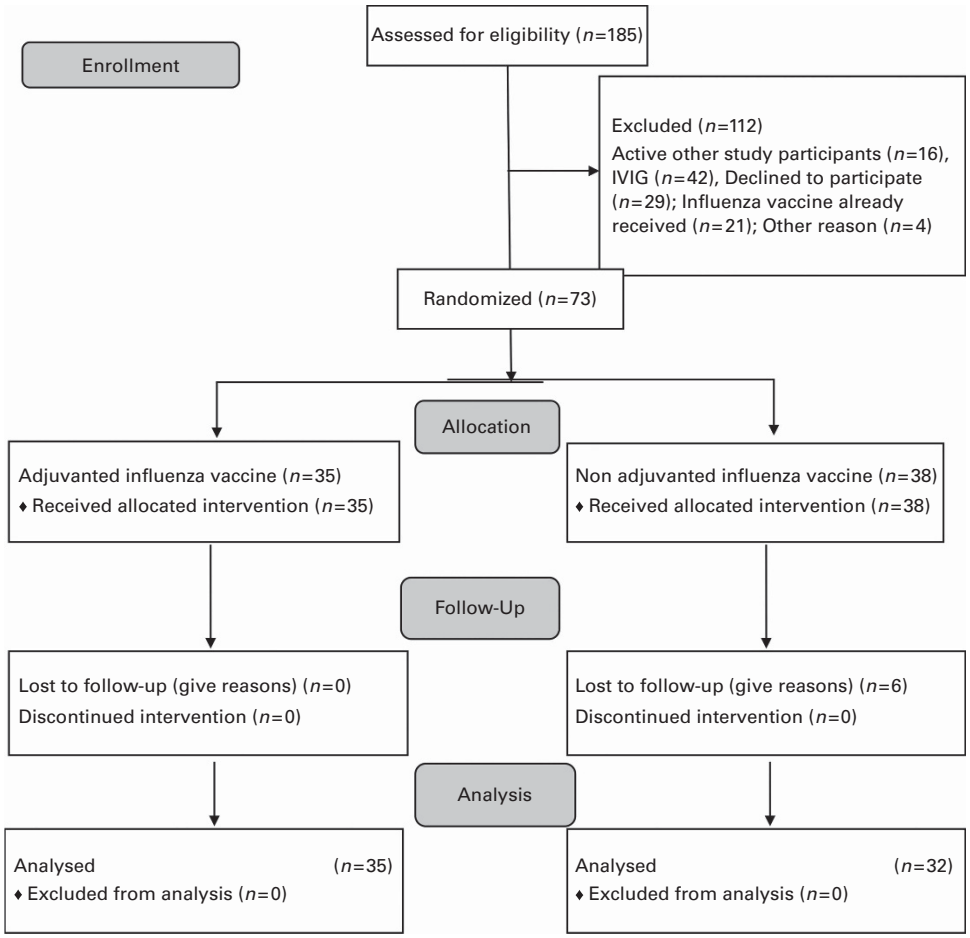

Figure 1. Consort diagram and study flow. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.

The adjuvanted vaccine was Fluad (Novartis, Canada) and the non-adjuvanted vaccine was Influvac (Abbott, Canada). Vaccines were administered by unblinded study team member to participants' non-dominant arm. Sera were obtained pre- and 4 weeks post vaccination for strain-specific antibody testing. Adverse events were assessed by a blinded study team member at 48 h and 7 days after vaccination to assess side effects. Adverse events were graded as mild (no interference in daily activities), moderate (some interference in daily activities) and severe (participants unable to perform daily activities). Secondary outcomes, such as influenza infection, hospitalization, death and GVHD were followed up to 6 months after vaccine administration. The study was registered at www.clinicaltrials.gov number NCT02560909.

Laboratory methods

Sera were stored at − 80 °C until the day of analysis. Laboratory staff were blinded to vaccine allocation and performed hemagglutination inhibition assay for three strains contained in the vaccine. This was done at the Public Health England, National Infection Service, Colindale, Health Protection Agency, UK using the procedure described elsewhere.[12] Titers were reported from below the lower limit of detection < 10 to 1:2048. Antibody concentrations that were below the lower limit of detection (< 10) were treated as 5 for the purpose of analysis.

Definitions and statistics

The following variables were used to assess vaccine immunogenicity: seroprotection was defined as a strain-specific titer of 1:40 or greater. Seroconversion was defined as a fourfold rise or greater in titer from baseline and achieved seroprotection. Seroconversion factor was calculated by dividing the post-immunization titer by the prevaccine titer. Geometric mean fold rise was calculated as the geometric mean of seroconversion factor. The sample size was calculated based on the previous studies using seasonal influenza vaccine in HSCT patients. Those studies indicated a response rate of approximately 30% to at least one of the three antigens in the vaccine. Therefore, our study would have 80% power to detect an increase of 30% in vaccine seroconversion for the per-protocol sample. The primary end point was defined as seroconversion to at least one of three influenza vaccine antigens. Previous acute GVHD was defined as any acute GVHD developing within 100 days after HSCT.[13] After 100 days, we recorded whether the patient had new onset GVHD requiring immunosuppressives or GVHD at a new site requiring additional immunosuppression. Demographics were analyzed using descriptive statistics. Pre- and post-vaccination titers were compared using Wilcoxon rank-sum test. Univariate analyses were performed to determine significant factors affecting seroconversion to at least one vaccine antigen using chi-squared or Fisher's exact test for categorical variables and Mann–Whitney *U* for continuous variables. For multivariate analysis, a model was constructed using variables that had a *P*-value less than 0.2 on univariate analysis. Multivariate analysis was performed using logistic regression. Statistical significance was defined as a *P*-value < 0.05. Statistical analysis was performed using IBM SPSS version 22.0 (Chicago, IL, USA) and GraphPad Prism version 7.0 (La Jolla, CA, USA).

RESULTS

Patient population

From October 2015 to January 2016, we screened 185 allogeneic HSCT patients (Figure 1). Of these, 112 patients were excluded for various reasons including use of IVIG ($n = 42$) and prior receipt of influenza vaccine ($n = 21$). Therefore, we enrolled 73 allogeneic stem cell transplant recipients (35 adjuvanted, 38 non-adjuvanted) and all of them received a study vaccine. Baseline characteristics of the cohort were similar between groups and details are shown in Table 1. The overall median time from transplant to vaccination

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Table 1. Patient characteristics at the time of enrollment

	Adjuvanted vaccine (N = 35)	*Non-adjuvanted vaccine (N = 38)*	P	*Total (N = 73)*
Age (years), median (range)	54.5 (23–74)	52.5 (22–69)	0.37	54 (22–74)
Gender (male/female)	23/12	17/21	0.072	
Baseline disease				
AML	13 (37.1%)	18 (47.4%)	0.063	31 (42.5%)
MDS	14 (40%)	6 (15.8%)		20 (27.4%)
Other	8 (22.9%)	14 (36.8%)		22 (30.1%)
Donor type				
Related	16 (45.7%)	16 (42.1%)	0.85	32 (43.8%)
Unrelated	18 (51.4%)	20 (52.6%)		38 (52.1%)
Cord blood	1 (2.9%)	2 (5.3%)		3 (4.1%)
Time from transplantation to vaccination (months), median (range)	19 (2–266)	10 (2–156)	0.67	12 (2–266)
Prior year vaccination (2014–2015)	14 (40%)	13 (34.2%)	0.60	27 (37%)
History of documented influenza in 2014–2015	3 (7.9%)	0 (0%)	0.09	3 (4.1%)
Previous acute GVHD	22 (62.9%)	26 (68.4%)	0.61	48 (65.8%)
Ongoing GVHD	22 (62.9%)	20 (52.6%)	0.37	42 (57.5%)
ATG within 1 year prior	3 (8.6%)	3 (7.9%)	0.91	6 (8.2%)
Immunosuppression				
Prednisone	16 (45.7%)	17 (44.7%)	0.93	33 (45.2%)
Cyclosporine	9 (25.7%)	7 (18.4%)	0.45	16 (21.9%)
Tacrolimus	1 (2.9%)	2 (5.3%)	0.60	3 (4.1%)
MMF/MPA	4 (11.4%)	5 (13.2%)	0.82	9 (12.3%)
Azathioprine	7 (20%)	6 (15.8%)	0.63	13 (17.8%)

Abbreviations: AML = acute myelogenous leukemia; GVHD = graft-versus-host disease; MDS = myelodysplastic syndrome; MMF/MPA, mycophenolate mofetil/mycophenolic acid.

was 380 days (range 85–8107). Other demographic characteristics including prior season influenza vaccination, presence of GVHD and immunosuppression at the time of vaccination were well-balanced in the two groups.

Vaccine immunogenicity

Of the 73 enrolled patients, 6 did not have either pre-vaccination or post-vaccination sera collected and were excluded from the immunogenicity analysis (Figure 1). Therefore, for the immunogenicity analysis, 67 patients were analyzed (35 adjuvanted, 32 non-adjuvanted). Geometric mean titers increased significantly from baseline after vaccination in both groups (Supplementary Figures 1a–c). Seroprotection rates after vaccination were similar in the two groups (Table 2). Cumulative distributions of post-immunization titers are shown in Supplementary Figures 2a–c. Seroconversion to at least one of three influenza vaccine antigens was present in 62.9% vs 53.1% in adjuvanted vs non-adjuvanted vaccine respectively ($P = 0.42$) (odds ratio (OR), 1.49; 95% confidence interval (95% CI), 0.56–3.96) (Figure 2). In the total cohort, baseline seroprotection (before vaccination) to A/H1N1, A/H3N2 and B was present in 32/67 (47.8%), 31/67 (46.3%) and 29/67 (43.3%), respectively, and was not significantly different between the two groups.

Factors affecting vaccine seroconversion were analyzed including time after transplantation, immunosuppression, prior influenza vaccination as well as age (Table 3). In univariate analysis, patients receiving calcineurin inhibitor (CNI) had a lower likelihood of seroconversion ($P < 0.001$). Longer time from transplant was associated with greater seroconversion ($P = 0.001$). There was also a trend to increased immunogenicity with adjuvanted vaccine in patients who were more than 6 months post transplant ($P = 0.06$) (Figure 3). Baseline seroprotection to an influenza antigen was not associated with greater seroconversion rates. However, receipt of prior season influenza vaccination was associated with greater seroconversion ($P = 0.004$). Prior season vaccine was given only to patients in the post-transplant period. For the multivariate analysis, all variables with *P*-value < 0.2 were

Table 2. Immunogenicity parameters of adjuvanted vs non-adjuvanted influenza vaccine

	Adjuvanted vaccine (N = 35)	*Non-adjuvanted vaccine (N = 32)*	P-*value*
GMT pre-vaccination			
A/H1N1	78.1	86.9	0.54
A/H3N2	85.1	51.6	0.48
B	124.0	140.6	0.22
GMT post-vaccination			
A/H1N1	319.6	195.9	0.34
A/H3N2	480.7	359.3	0.27
B	298.9	240.5	0.97
Seroprotection post-vaccine			
A/H1N1	20 (57.1%)	19 (59.4%)	0.85
A/H3N2	25 (71.4%)	23 (71.9%)	0.97
B	20 (57.1%)	22 (68.8%)	0.32
Seroconversion			
A/H1N1	11 (31.4%)	7 (21.9%)	0.38
A/H3N2	20 (57.1%)	13 (40.6%)	0.18
B	13 (37.1%)	8 (25.0%)	0.29
Geometric mean seroconversion factor			
A/H1N1	2.58	2.00	0.41
A/H3N2	4.33	3.51	0.57
B	2.97	1.92	0.15

Abbreviation: GMT = geometric mean titer.

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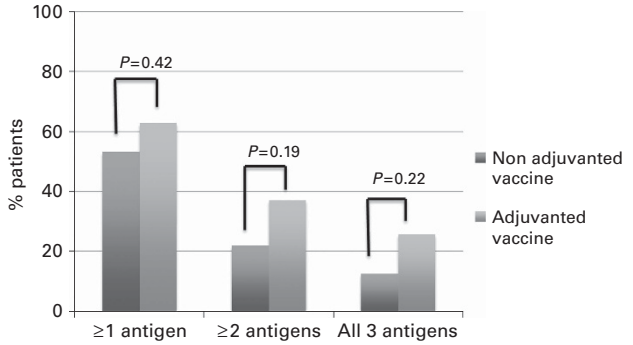

Figure 2. Seroconversion to at least one, two or all three vaccine antigens based on vaccine type. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.

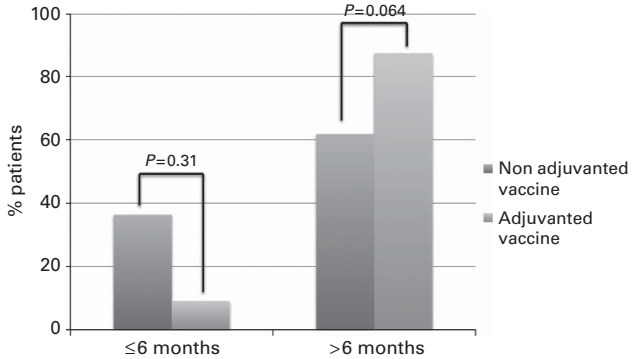

Figure 3. Seroconversion rates in patients at 6 months or earlier post transplant and those beyond 6 months. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.

included (age, ATG use, prior influenza vaccine, CNI use, MMF/MPA use and time from transplant). We noted that CNI use and time from transplant were highly related variables (i.e. as time from transplant increased, CNI use declined, $P = 0.006$).

Therefore, we created two models: one including CNI use and the other including time from transplant. These variables were significantly associated with poor seroconversion in their individual models, that is, time from transplant (OR 1.002, 95% CI 1.000–1.004, $P = 0.047$) and CNI use (OR 9.28, 95% CI 1.85–46.51, $P = 0.007$; Table 3).

Vaccine safety

Vaccine-related adverse events were assessed in the 73 patients who received study vaccine. Within 7 days of immunization, there were no statistically significant differences for local and systemic side effects in adjuvanted and non-adjuvanted groups. Local side effects including erythema, induration and tenderness were present in up to 2.9% of patients. Febrile reactions occurred in 5.7% vs 2.6% of patients in the adjuvanted and non-adjuvanted groups, respectively. In addition, new onset GVHD, or GVHD at a new site, occurred in 9/35 (25.7%) vs 10/38 (26.3%) patients in the adjuvanted and non-adjuvanted groups respectively ($P = 0.95$). Over the 6-month follow-up period, there were 19 hospitalizations (8/35 in adjuvanted and 11/38 in non-adjuvanted vaccine group, $P = 0.60$), none related to vaccination. There were three deaths in the cohort unrelated to vaccination. Documented influenza infections were seen in a total of 8 (11.0%) participants (five in adjuvanted and three in non-adjuvanted groups). The median time from vaccination to influenza infection in these patients was 121.5 (range 40–169) days. Three patients developed influenza A/HINI infection. Of these three, one patient achieved both seroprotection and seroconversion to the vaccine A/H1N1 strain. One patient developed A/H3N1 infection and this patient had no vaccine response. Four patients developed influenza B infection. Of these four patients, two had achieved seroprotection and one had achieved seroconversion. However, all cases were mild with no requirement for hospitalization.

DISCUSSION

We performed a randomized controlled trial using adjuvanted vs non-adjuvanted influenza vaccine in a cohort of allogeneic HSCT recipients. We found that the two vaccines performed similarly with regard to immunogenicity and the seroconversion rates ranged from 21.9 to 57.1% depending on the influenza strain. In the total cohort we found that time from transplant and use of calcineurin inhibitors were significant factors in determining vaccine immunogenicity. Conversely, receipt of prior influenza vaccine had a beneficial effect in vaccine response. Both vaccines were safe and well tolerated.

Studies with MF59 adjuvanted influenza vaccine in the $\geqslant$65-year-old population have shown increased immunogenicity compared with standard inactivated vaccine.[14,15] For example, Seo *et al.*[15] showed that the GMTs for A/H3N2 in the adjuvanted vaccine group were higher than those in the non-adjuvanted vaccine group although no significant differences in immunogenicity were seen with A/H1N1 or B strains. To our knowledge, there are no previous published studies of MF59-adjuvanted influenza vaccine in HSCT recipients and there are limited data for this vaccine in other immunocompromised groups. We previously performed a randomized trial comparing the MF59-adjuvanted vaccine with standard vaccine in adult kidney transplant patients. Although the vaccines were similar in the primary analysis, an *a priori* subgroup analysis of patients aged 18–65 years showed that the adjuvanted vaccine had comparatively greater immunogenicity.[16]

Although there are no previous data with MF59 adjuvant in HSCT recipients, a number of investigators have studied the ASO3-adjuvanted pandemic influenza vaccine.[17] Similar to MF59, AS03 is also an oil-in-water emulsion. This AS03 adjuvanted pandemic vaccine contained only 3.75 μg of antigen compared with the 15 μg used in the current study. Using this vaccine, studies found seroprotective titers ranging from 44 to 51%.[7,18,19] Engelhard *et al.* found that there was a significant rise in titers after a second dose of vaccine. Similarly, de Lavallade *et al.*[18] studied 97 allogeneic HSCT recipients and noted a seroprotection rate of 46% with one dose which increased to 73% after a second dose. The increase in vaccine response after the second dose may have been specific to the pandemic vaccine as has not been previously seen in seasonal vaccine studies. Our seroprotection rates range from 57.2 to 71.9%, greater than those noted in previous studies. This may be due to a significant proportion (37%) of the population who received influenza vaccine in the prior season as well as a greater amount of antigen in the vaccine. Immunization in the previous season was significantly associated with seroconversion to vaccine antigens.

We also found that there was little response in patients less than 6 months from transplant and that time from transplant significantly impacted vaccine response. This is similar to previous studies in HSCT patients that found that time from transplant was an important factor in vaccine immunogenicity.[5,7,19,20] Despite the poor reconstitution in humoral immunity in the early post-transplant period, influenza vaccine may still be beneficial. One study has shown a cellular response could still occur despite the lack of humoral response.[21] A novel finding in our study was that use of CNI was detrimental to vaccine immunogenicity. CNIs inhibit T-cell function which is important in vaccine responses.

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Table 3. Univariate analysis of factors associated with seroconversion to at least one influenza antigen

Variable	*Seroconversion* (n = *39*)	*No seroconversion* (n = *28*)	*Odds ratio (95% CI)*	P-*value*
Age	51 (22–74)	58.5 (24–68)	0.97 (0.94–1.01)	0.15
Male gender	20 (51.3%)	18 (64.3%)	1.71 (0.63–4.63)	0.29
ATG within 1 year	1 (2.6%)	5 (17.9%)	0.12 (0.013–1.10)	0.075
Prior influenza vaccine	19 (48.7%)	4 (14.3%)	5.70 (1.67–19.5)	0.004
Prednisone	20 (51.3%)	11 (39.3%)	1.63 (0.61–4.36)	0.33
CNI	3 (7.7%)	15 (53.6%)	0.07 (0.018–0.29)	< 0.001
MMF/MPA	7 (17.9%)	1 (3.6%)	5.91 (0.68–51.1)	0.13
Azathioprine	9 (23.1%)	4 (14.3%)	1.80 (0.49–6.57)	0.53
Time from transplant (months) median (range)	27 (2–266)	4 (2–43)	1.003 (1.001–1.005)	0.001
Adjuvanted vaccine	22 (56.4%)	13 (46.4%)	1.49 (0.56–3.96)	0.42
Concurrent GVHD	25 (64.1%)	14 (50%)	1.79 (0.67–4.80)	0.25

Abbreviations: ATG = antithymocyte globulin; CNI = Calcineurin inhibitor; GVHD = graft-versus-host disease; MMF/MPA = mycophenolate mofetil/mycophenolic acid.

We found that four out of eight patients who developed influenza had achieved seroprotective titers to influenza vaccination. This may be due to rapidly waning immunity or the possibility that the widely accepted seroprotective titer of 1:40 is not adequate for influenza protection in this population. Although the patients contracted influenza, the infection was mild as there was no need for hospitalization.

Other strategies to improve influenza vaccine immunogenicity have been attempted in HSCT recipients. Karras *et al*.[20] performed a randomized trial of one dose vs two doses of influenza vaccine in allogeneic HSCT recipients and showed a 19–32% seroprotection rate. However, there was no significant benefit from the second dose. More recently, Halasa *et al*.[22] showed that the high-dose influenza vaccine (containing 60 μg of antigen) had greater immunogenicity than standard dose vaccine in 44 adult HSCT recipients especially for A/H3N2. In addition, based on the study from Ambati *et al*.,[23] pre-transplant vaccination may improve influenza protection in the post-transplant period. Further trials are needed to find an optimal strategy.

Our study has some limitations. The sample size was limited and the primary end point was not met reducing the statistical power of the study. We were not able to enroll a larger sample size with a significant reason being the number of patients receiving monthly IV immune globulin therapy. These patients were excluded as IgG measurements were the primary end point and IVIG use may increase the amount of antibody measured pre- and post-vaccination. Our study also examined vaccine immunogenicity as a primary end point rather than the clinical end point of influenza infection. Although the latter end point would be preferable, a significantly larger sample size would be required. However, immunogenicity parameters are commonly used to annually license influenza vaccines. The MF59 influenza vaccine was authorized for use in persons $\geqslant 65$ years based on comparable immunogenicity to non-adjuvanted vaccine rather than efficacy studies. Although we were able to detect a difference in immunogenicity for those who received CNI, we could not show the same for MMF/MPA likely due to low number of patients that were receiving this immunosuppressive.

In conclusion, adjuvanted vaccine had similar immunogenicity and safety as compared with non-adjuvanted vaccine in adult allogeneic HSCT recipients. Therefore, our study suggests that MF59-adjuvanted vaccine could be safely used in HSCT recipients as a potential means of improving immunogenicity and should be evaluated further.

CONFLICT OF INTEREST

DK has received research funding from Roche and GSK, honoraria from GSK and Sanofi. The remaining authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

YN, AH and DK participated in research design, the writing of the paper, the performance of the research and data analysis. JL and DDHK participated in writing of the paper and data analysis. PA and KH participated in the performance of the research.

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