Supplementary Appendix

Supplement to: Schuster JE, Hamdan L, Dulek DE, et al. Influenza vaccine in pediatric recipients of hematopoietic-cell transplants. N Engl J Med 2023;388:374-6. DOI: 10.1056/NEJMc2210825

This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

Table of Contents

The members of the Pediatric SCT Flu Study Group

Children's Mercy Kansas City: Rakesh Goyal

Vanderbilt University Medical Center: Rendie McHenry, Margaret Bender, Shari Barto

Children's Hospital of Philadelphia: Michael Russo

Nationwide Children's Hospital: Lauren Shoemaker

University of California San Francisco Benioff Children's Hospital: Kenny Truong, Christopher Dvorak

St. Jude Children's Research Hospital: Kim J. Allison

Baylor School of Medicine, Texas Children's Hospital: Swati Naik, Christopher Williams, Texas Children's Hospital Research Resources Office

Cincinnati Children's Hospital: Samantha Blum

Seattle Children's Research Institute: Kirsten Lacombe, Hannah Smith

Methods

TRIAL DESIGN AND PARTICIPANTS

This multi-center double-blinded phase II randomized controlled influenza vaccine trial of pediatric HCT recipients was conducted over three influenza seasons (2016-17, 2017-18 and 2018-19) at nine U.S. study sites: Vanderbilt University Medical Center (TN); University of California San Francisco Benioff Children's Hospital – San Francisco (CA), Children's Mercy Kansas City (MO), Cincinnati Children's Hospital (OH), Nationwide Children's Hospital (OH), Children's Hospital of Philadelphia (PA), St. Jude Children's Research Hospital (TN), Texas Children's Hospital (TX), and Seattle Children's Hospital (WA).

Eligible participants were 3 to 17 years old and 3 to 35 months post-allogeneic HCT. Participants with graft versus host disease (GVHD) were eligible if their disease and GVHD therapy were stable for at least four weeks prior to enrollment. Participants were eligible to re-enroll for a maximum of one subsequent influenza season following initial enrollment; data from the repeated year are not included in this analysis. Further details regarding inclusion and exclusion criteria and the schedule of events are provided in the clinical protocol.

Participants were randomized 1:1 to receive either two doses of HD-TIV or SD-QIV with a target interval of 28-42 days between vaccine doses (at the time of this study, the high-dose formulation of the quadrivalent vaccine was not available). Randomization was blocked and stratified by site and time post-HCT. Moreover, randomization of participants 3 to 11 months post-HCT was stratified by GVHD status, systemic steroid use, or receipt of alemtuzumab or anti-thymocyte globulin pre-transplant, cord blood or haploidentical transplant, or post-HCT cyclophosphamide. For participants 12 to 35 months post-HCT, randomization was stratified by GVHD and/or systemic steroid use.

The study was reviewed and approved by the Institutional Review Board (IRB) at each of the study sites. All parents/guardians provided written informed consent; assent was confirmed at IRB site-specific ages. Study data were collected and managed using REDCap electronic data capture tools hosted at Vanderbilt University Medical Center.

SAMPLE SIZE AND POWER

Our study's power calculations were based on an analysis of a difference in proportions of patients in each vaccine group achieving a titer ≥1:40, as the variability in titer outcomes was not well understood at the time. We determined that a total of 160 evaluable participants would be necessary to achieve 80% power to detect a 20% difference in proportions (initial hypothesis: 40% in HD-TIV relative to 20% in SD-QIV). However, anticipating a 20% dropout rate, we set the initial target enrollment goal to 200.

VACCINE

Vaccines were provided by Sanofi (Swiftwater, PA) and investigational pharmacies at each site dispensed study vaccines per randomization code in a blinded manner. SD-QIV contained 15μg hemagglutinin from each strain (A/H1N1, A/H3N2, B/Victoria, B/Yamagata). HD-TIV contained 60μg of the hemagglutinin from each strain except for B/Yamagata.

STUDY PROCEDURES

Vaccines were administered as 0.5mL intramuscular deltoid injections given at a target interval of 28-42 days apart (visits 1 and 2). Per protocol, serological assays were scheduled for

collection prior to administration of each vaccine (visits 1 and 2) and 28-42 days after dose 2 (visit 3).

SAFETY EVALUATIONS

Parents and/or participants recorded local and systemic reactions using a memory aid for seven days after each vaccine. Reactions were graded according to a mild/moderate/severe toxicity scale and entered into REDCap. Grade three or higher unsolicited adverse events and severe adverse events (SAE) were also collected (*see clinical protocol for details of grading scales).*

IMMUNOGENICITY ASSAYS

Sera were frozen at each site, shipped to Vanderbilt, and then bulk-shipped to Sanofi Global Clinical Immunology for blinded HAI testing for each vaccine-specific antigen.[1] When blood volume was insufficient, HAI testing of influenza A antigens was prioritized.

STATISICAL ANALYSES

Baseline descriptive statistics were reported as median (IQR) for continuous variables or absolute and relative frequencies for categorical variables. All descriptive analyses were based on participants receiving at least one dose. If participants received an incorrect dose, reactogenicity outcomes and HAI titers were treated as missing.

The aGMR (HD-TIV/SD-QIV) was estimated using linear mixed models with log-transformed HAI titer, with adjustments for, age, log-transformed baseline titer, time post-HCT, $CD4^+$ count, $CD19^+$ count, absolute lymphocyte count (ALC), GVHD, and malignancy, and with participant- and site-specific random effects. The aGMR to B/Victoria was evaluated as a secondary endpoint; B/Yamagata was analyzed as a control since this strain was included in SD-QIV but not in HD-TIV. Missing data (including missing values resulting from incorrect vaccine doses) were addressed using multiple imputation by chained equations (M=300 iterations). The following variables were included in the imputation model: dose group, study site, sex, continuous age, race/ethnicity, continuous time post-transplant, stem cell source, control prep regimen, indicators of related donor, GVHD at baseline, and malignancy, baseline lab values ($CD4^+$, $CD8^+$, $CD19^+$, ALC, and absolute neutrophil count [ANC]), and HAI titers at all time points. A total of two participants died during the follow-up period following the second dose (i.e., prior to visit 3); their observations were included in analyses, though missing values for variables due to death were not imputed.

The primary safety endpoint, reactogenicity, was summarized as frequency of injection site (swelling, erythema, tenderness, and pain) and systemic reactions (fever [defined as ≥38.0 ℃], decreased activity, myalgia, nausea, headache, fatigue, and vomiting) within the seven-day periods following each of the vaccines.

To assess model fit, we examined residual-versus-fitted plots and correlation matrices to ensure sufficiency.

Figure S1. Enrollment, randomization, and vaccine status. A total of 180 participants were consented, among whom 170 were subsequently randomized and vaccinated. Among the 85 participants randomized to receive SD-QIV, 80 (94%) received both doses; among the 85 participants randomized to receive HD-TIV, 83 (98%) received both doses. In addition, one subject was randomized but did not consent (not depicted on diagram).

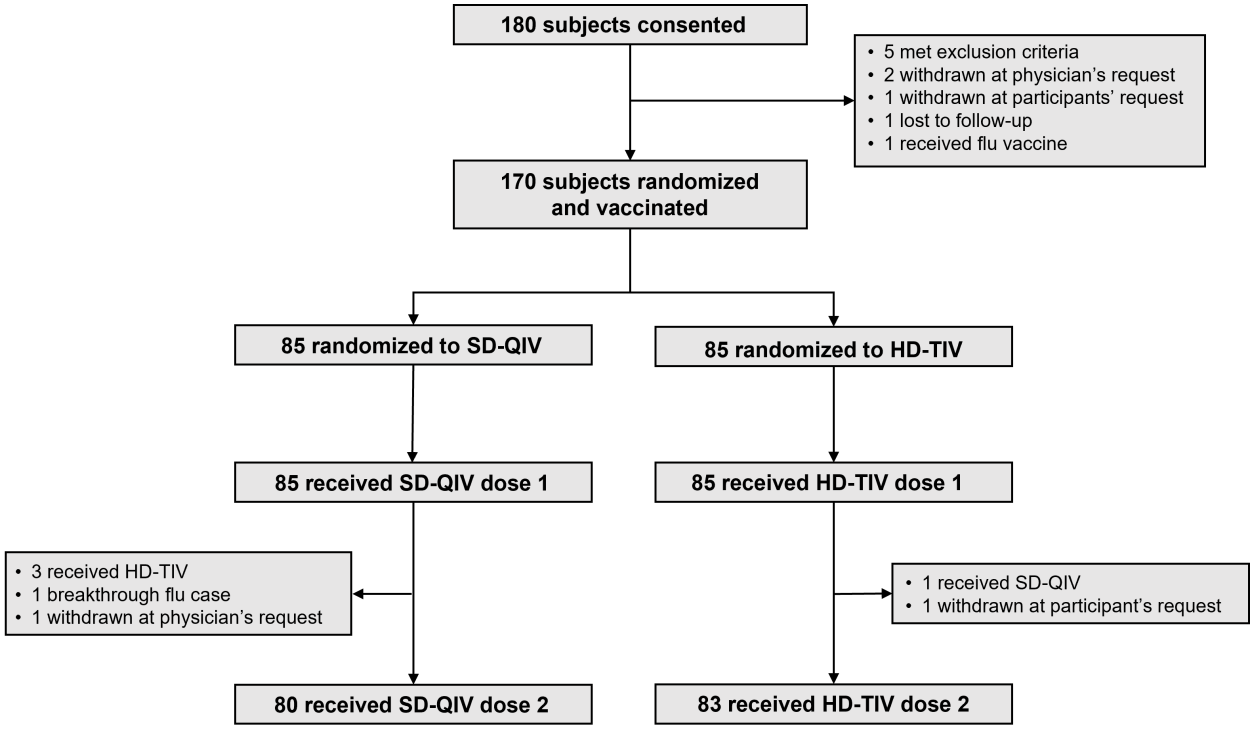

Figure S2. Injection-site and systemic reaction frequencies. Displayed are the relative frequencies of each injection site and systemic reaction type for each vaccine group (SD-QIV vs. HD-TIV) following each dose. Reactions were further graded according to a mild/moderate/severe toxicity scale (grades 1 through 3, respectively), which are additionally marked by shading.

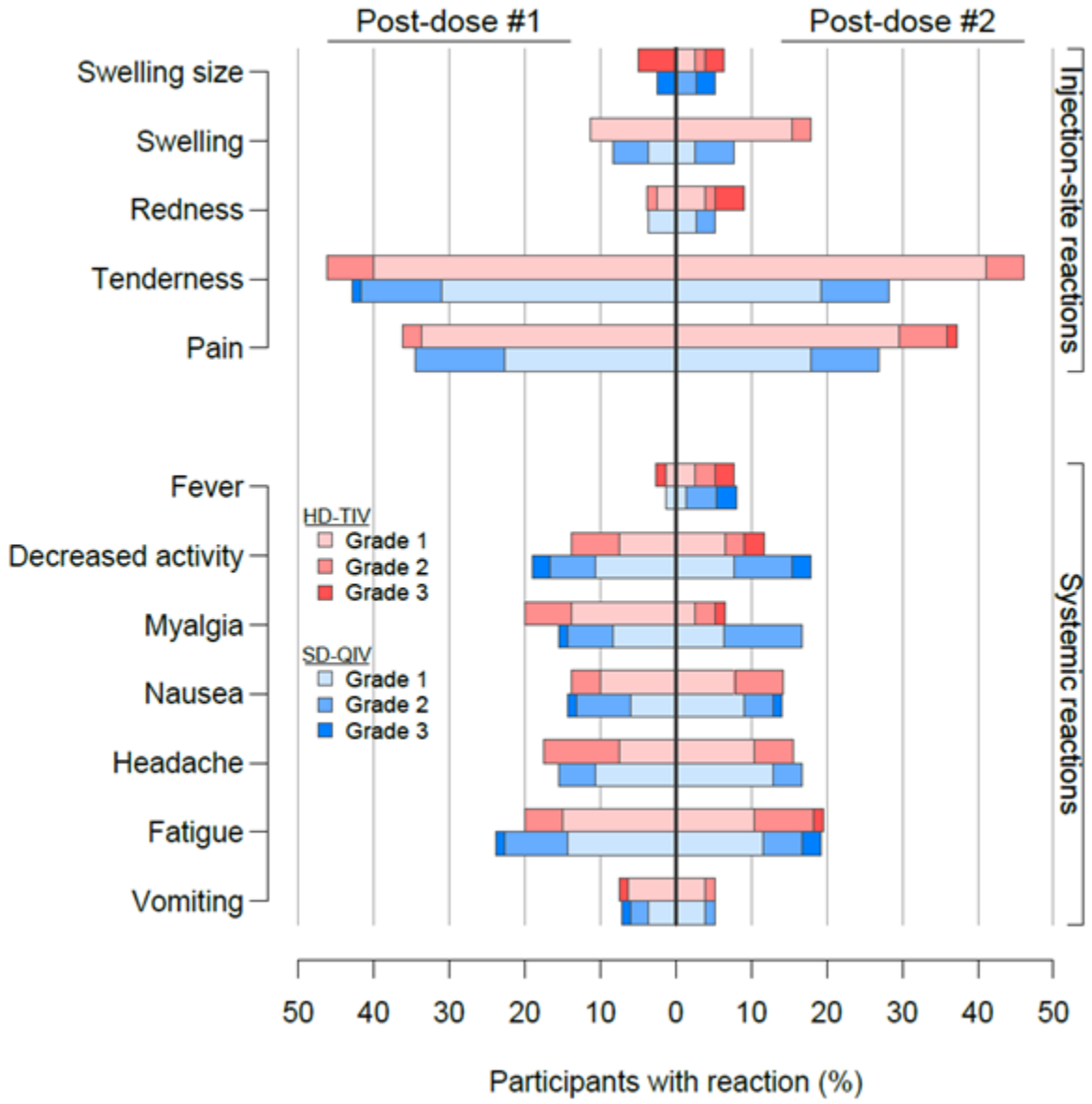

Table S1. Demographics of enrolled participants.

	All ***N*=170**	**SD-QIV** ***n*=85**	**HD-TIV** ***n*=85**
Age at enrollment (years)			
Mean (SD)	10.8 (4.3)	10.6 (4.4)	11.0 (4.2)
Median (IQR)	10.9 (7.0, 14.3)	10.6 (7.0, 14.2)	11.8 (7.1, 14.3)
Minimum, maximum	3.1, 18.0	3.1, 18.0	3.3, 18.0
Male—*n* (%)	94 (55.3)	49 (57.7)	45 (52.9)
*Race—*n* (%)			
White	117 (68.8)	62 (72.9)	55 (64.7)
Black/African American	31 (18.2)	12 (14.1)	19 (22.4)
Asian	6 (3.5)	2 (2.4)	4 (4.7)
American Indian/Alaskan Native	1 (0.6)	1 (1.2)	0 (0)
Other/unknown	15 (8.8)	8 (9.4)	7 (8.2)
*Hispanic—*n* (%)	36 (21.2)	17 (20.0)	19 (22.4)
Months from transplant to enrollment—median (IQR)	7.8 (4.3, 13.4)	9.2 (5.0, 15.9)	6.0 (4.1, 12.2)

N: Number of participants enrolled who received at least one vaccination; SD: standard deviation; IQR: interquartile range. *Race and ethnic group were reported by parents or guardians.

Table S2. Geometric mean titers (GMT) and 95% confidence intervals (95% CIs) by study group and adjusted geometric mean ratio (aGMR) and 95% CIs after the second vaccine dose.

	GMT (95% CI) SD-QIV	GMT (95% CI) HD-TIV	aGMR (95% CI) (HD-TIV/SD-QIV)
A/H1N1	167 (116–241)	275 (196–387)	1.65 (1.06–2.57)
A/H3N2	235 (158–351)	327 (208–515)	2.11 (1.32–3.38)
B/Victoria	188 (131–270)	225 (154–329)	1.46 (0.93–2.31)
B/Yamagata*	269 (190–382)	97 (71–133)	0.40 (0.26–0.63)

* B/Yamagata is not included in HD-TIV.

Acknowledgments

We thank Maggie Morrison Jones, Marsha Dunn, Brynn Szeles, Erin Yepsen, Claudia Guevara Pulido, Ying Lu, and Sandra Alvarez-Macias. We also thank the patients and their families as well as the members of the data safety monitoring board.

Reference

1. Halasa NB, Savani BN, Asokan I, et al. Randomized Double-Blind Study of the Safety and Immunogenicity of Standard-Dose Trivalent Inactivated Influenza Vaccine versus High-Dose Trivalent Inactivated Influenza Vaccine in Adult Hematopoietic Stem Cell Transplantation Patients. Biol Blood Marrow Transplant 2016;22(3):528-35. DOI: 10.1016/j.bbmt.2015.12.003.