Supplementary Appendix

Supplement to: Hughes BL, Clifton RG, Rouse DJ, et al. Randomized trial of hyperimmune globulin for congenital CMV infection — 2-year outcomes. N Engl J Med 2023;389:1822-3. DOI: 10.1056/NEJMc2308286

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

Supplementary Appendix

Table of Contents

Trial Sites and Study Personnel 2
Acknowledgement 3
Statistical Methods 4
Sensitivity Analyses 4
Figure S1. Screening, enrollment, randomization, and follow-up 6
Table S1. Representativeness of study participants 7
Table S2: Sensitivity analysis of outcomes using multiple imputation 8
Figure S2. Tipping point analysis of composite outcome 8

Trial Sites and Study Personnel

Each clinical center consisted of one or more additional performance sites as listed below. Also listed are members of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network who contributed to the research in addition to the authors.

Clinical Centers:

Brown University, Providence, RI – B. Hughes, D. Rouse, D. Allard, E. Werner, J. Rousseau, L. Beati, J. Milano, J. Lee

University of Texas Medical Branch, Galveston, TX – G. Saade, A. Salazar, L. Pacheco, J. Patel, D. Carlson, K. Smith, A. Nounes, J. DeVolder

Northwestern University, Chicago, IL – G.Mallett, W.Grobman, A. Peaceman

NorthShore University Health System, Evanston, IL – M. Dinsmoor, K. Paycheck

Columbia University, New York, NY – C. Gyamfi-Bannerman, S. Bousleiman, R. Wapner V. Carmona, M. Talucci

Christiana Care, Wilmington, DE – M. Hoffman, A. Vanneman

St. Peter's University Hospital, New Brunswick, NJ – K. Palomares, C. Perez

Drexel University, Philadelphia, PA – L. Plante, C. Tocci,

New York Presbyterian Queens, Flushing, NY – D. Skupski R. Chan-Akeley

Lehigh Valley Hospital, Allentown, PA

University of Utah Health Sciences Center, Salt Lake City, UT – M. Varner, K. Hill, A. Sowles

LDS Hospital, Salt Lake City, UT – C. Meadows

McKay-Dee Hospital, Ogden, UT – S. Dellerman

Intermountain Med Center, Salt Lake City, UT – L. Hansen, S. Esplin

University of North Carolina at Chapel Hill, Chapel Hill, NC – W. Goodnight, K. Clark, J. Thorp, S. Timlin

WakeMed Health & Hospitals, Raleigh, NC – C. Beamon, H. Byers

Prisma Health, Greenville, SC – K. Eichelberger, A. Moore

University of *Alabama at Birmingham, Birmingham, AL* – A. Tita, S. Harris, L. Harper, J. Biggio, M. Parks, J. Sheppard

The Ohio State University, Columbus, OH– M. Costantine, A. Bartholomew, M. Landon, J. Iams, C. Shellhaas, K. Markham, B. Rink, C. Buhimschi, F. Johnson, L. Webb

Wright State University Miami Valley Hospital, Dayton, OH – D. McKenna, K. Fennig, K. Snow

Duke University, Durham, NC – G. Swamy, T. Bishop, J. Ferrara

University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO – R. Gibbs, K. Hale, K.D. Heyborne, J. Phipers

MetroHealth Medical Center-Case Western Reserve University, Cleveland, OH – E. Chien, W. Dalton

University Hospitals, Cleveland, OH – D. Hackney, A. Mayle

UT Health- University of Texas Health Science Center at Houston, Children's Memorial Hermann Hospital, Houston, TX – S. Chauhan, F. Ortiz, B. Sibai

Stanford University, Stanford, CA – Y. El-Sayed, C. Willson, N. Aziz, D. Lyell, A. Girsen, K. Sherwi

University of Texas Southwestern Medical Center, Dallas, TX – B. Casey, L. Moseley, J. Price, T. Thomas, L. Fay-Randall, A. Sias, M. Garcia

University of Pennsylvania, Philadelphia, PA – S. Parry, J. Craig

University of Pittsburgh, Pittsburgh, PA – H. Simhan, M. Bickus, F. Facco, M. Birsic

Madigan Army Medical Center, Joint Base Lewis-McChord, Tacoma, WA – P. Napolitano, L. Imbruglio, E. Hemman, J. Pates, L. Foglia

Data Coordinating Center: The George Washington University Biostatistics Center, Washington, DC – E. Thom, R. Clifton, L. Fete, L. Mele, V. L. Flowers-Fanomezantsoa, T. Boekhoudt, C MacPherson

Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD – U. Reddy, C. Spong, S. Pagliaro

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Statistical Methods

The original sample size of 800 pregnant people (400 in each group) was calculated to ensure at least 90% power to detect a 30% reduction in the primary outcome of congenital CMV infection (from 32% in the placebo group to 26.6% in the hyperimmune-globulin group), assuming a type I error (two-sided) of 5%. Before the trial began, it was estimated that the rates of the outcomes in the ordinal variable of overall child status at 2 years of age would be 11.7%, 5.5%, 21.6% and 61.2%. Assuming a 10% termination of pregnancy or fetal loss rate and 32% congenital infection rate, and further assuming that of the 32% of infected infants, 6% would die and 19% would have a severe disability, adjusting for a 5% lost to follow-up rate and a test for trend using a linear rank score, a sample size of 760 subjects was sufficient to detect at least a 30% reduction in each of the ordinal overall child status variable outcomes at age 2 years (11.7% to 7.8%, 5.5% to 3.7%, and 21.6% to 14.4%) with a power greater than 90 percent and type-I error (2-sided) of 5%. The Data and Safety Monitoring Committee recommended trial termination in June 2018 due to futility with 399 subjects enrolled, diminishing the planned trial power to detect differences in the ordinal variable outcome.

Analyses were performed according to the intention-to-treat principle. The effect for binary variables, such as the composite outcome, were estimated as an unadjusted relative risk with Wald confidence intervals, while continuous outcome variables were compared by reporting mean differences with pooled 95% confidence intervals. As this letter reports secondary outcomes, confidence interval widths have not been adjusted for multiplicity and should not be used for hypothesis testing.

Sensitivity Analyses

While partial outcome data was available on 90% of the children (**Figure S1**), there was a substantial amount of missing data for the composite outcome. This was primarily due to the difficulty of obtaining complete data for the hearing and developmental testing among young children. A high proportion of missing data has the potential to introduce bias in addition to reducing the power of the study. As a result, sensitivity analyses were performed to quantify the sensitivity of results to data not missing at random.

The first sensitivity analysis imputed the individual elements of the composite outcome and then computed the composite outcome based on the imputed components to leverage correlation across components of the composite outcome. The imputed datasets were analyzed using the same approach currently used for the complete case analysis (unadjusted relative risks or risk differences and confidence intervals). A total of 26 imputed datasets were created and an analysis was conducted on each one. After pooling the results from this approach, the estimated treatment effect was very similar to the same analysis including only complete cases (**Table S2**).

The second sensitivity analysis used a tipping point approach. For pairwise combinations of probabilities (ranging from 0 to 1, or equivalently from 0% to 100%) of observing the outcome among the participants with missing values (n=57 in the hyperimmune globulin group, and n=44 in the placebo group), the chi-squared test assessed the association between group and outcome. The results are presented in **Figure S2**, with yellow regions corresponding to combinations yielding p-values < 0.05, and red regions to combinations with p-values > 0.05. For example, assuming none of the 57 participants with missing values in the Active group actually experienced the outcome (i.e., probability of success equal to 0, first row), for the group

difference to become statistically significant at level 0.05 one would need 40% of the 44 participants in the Placebo group to experience the outcome. Overall, for the results to be statistically significant at level 0.05 the probability of an outcome among the participants with missing values in the placebo group would have to be more than 40% higher than the probability of an outcome among the participants with missing values in the hyperimmune globulin group. Equivalently, this corresponds to odds ratios higher than 9, in favor or the treatment group, which is extremely unlikely.

Figure S1. Screening, enrollment, randomization, and follow-up

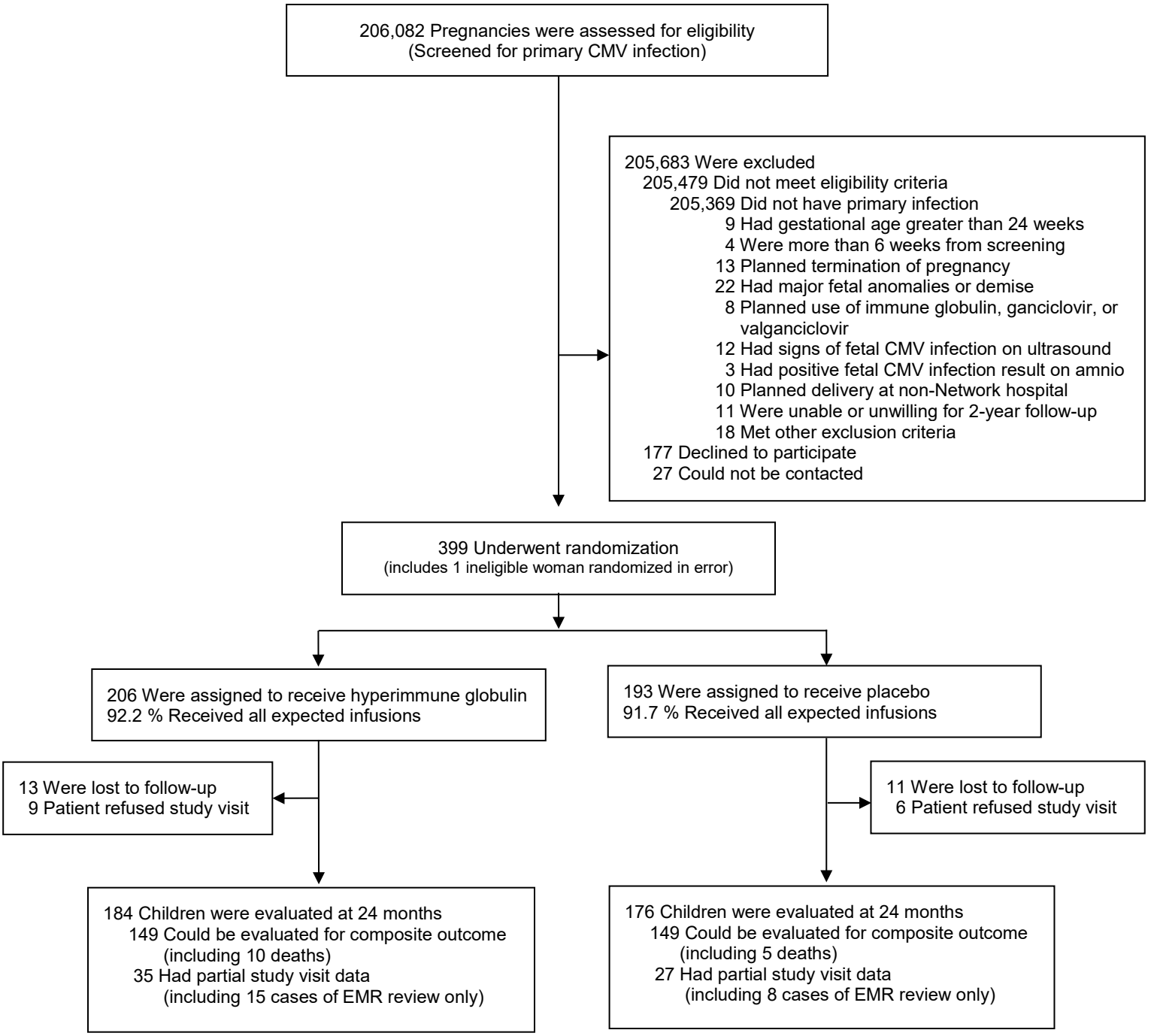


Table S1. Representativeness of study participants

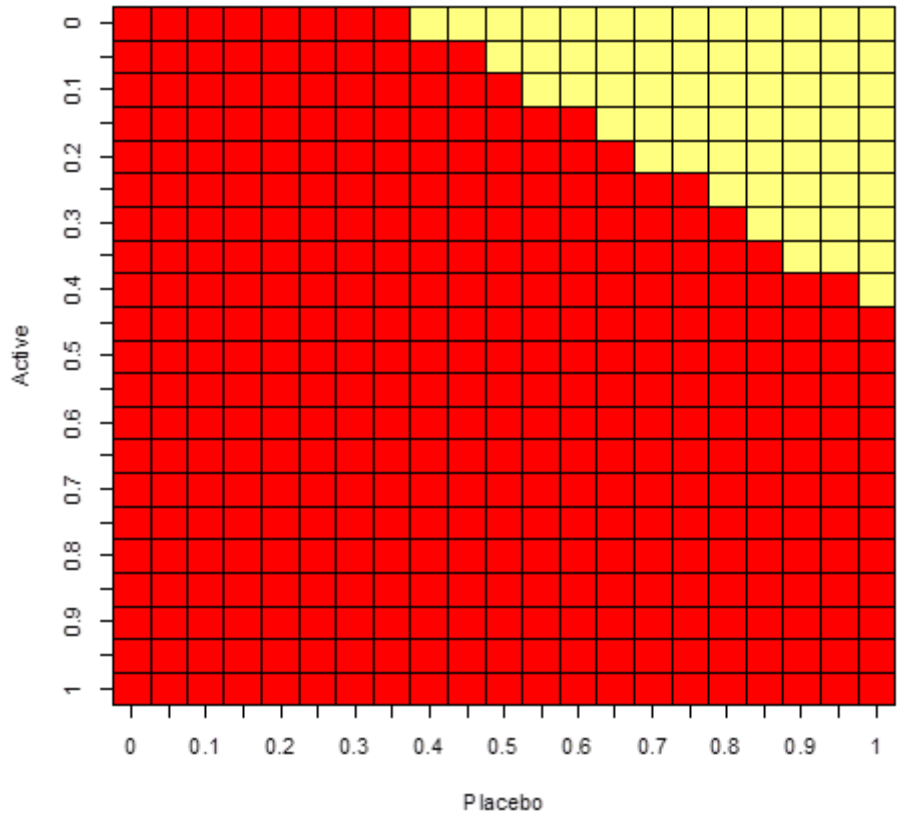
Condition under investigation	Congenital Cytomegalovirus (CMV)
Sex and gender considerations	Congenital Cytomegalovirus affects both male and female neonates.
Age	Congenital CMV affects only neonates
Race or ethnic group	Congenital CMV tends to be more common among Black individuals in the United States.
Geography	Incidence and severity of congenital CMV varies worldwide based on the CMV seroprevalence of the birthing population.
Other considerations	Throughout the world, CMV disproportionately impacts low-income communities.
Overall Representativeness of this trial	At birth, 48% of the infants were male and 52% were female. The mean age in our two-year follow-up cohort was 27 months. 65% of the study population were non-Hispanic white, 16% non-Hispanic black, 16% Hispanic, and 3% were categorized as other or unknown. The primary study enrolled at sites across the United States with variability in the seroprevalence of CMV in the birthing population.

Table S2: Sensitivity analysis of outcomes using multiple imputation

	Relative Risk or Risk Difference (95% CI)†	
	Complete case analysis	Imputation analysis N=399
Composite outcome*	1.33 (0.71, 2.50)	1.27 (0.70, 2.31)
Bayley Scale cognitive score	-0.9 (-4.1, 2.3)	-0.9 (-3.9, 2.10)
Bayley Scale motor score	-3.2 (-6.7, 0.3)	-2.6 (-6.2, 0.9)
< 10th percentile weight	0.94 (0.53, 1.67)	0.95 (0.55, 1.64)

*Composite outcome was calculated after individual elements of the composite outcome were imputed. †Confidence interval widths have not been adjusted for multiplicity and should not be used for hypothesis testing.

Figure S2. Tipping point analysis of composite outcome



For pairwise combinations of probabilities (ranging from 0 to 1, or equivalently from 0% to 100%) of observing the outcome among the participants with missing values (n=57 in the Active group, and n=44 in the Control group), the chi-squared test assessed the association between group and outcome. The results are presented above, with yellow regions corresponding to combinations yielding p-values < 0.05, and red regions to combinations with p-values > 0.05.