Long Term Immune Memory Responses to Human Papillomavirus (HPV) Vaccination Following 2 Versus 3 Doses of Quadrivalent HPV Vaccine

(Merck08 Study)

Clinical Trial Registration Number: NCT02968420

Date: 15 March 2019

Data Analysis Plan For Demographic/Clinical Characteristics and Serology Data

Objective: To compare the serum antibody responses to HPV 6, 11, 16 and 18 at 7 and 30 days post-booster dose of HPV vaccine in females that received either a primary 2 or 3 dose series of Q-HPV vaccine 8-10 years ago.

Primary testing is to determine non-inferiority (95% CI, lower bound >0.5) of geometric mean titer (GMT) ratios for anti-HPV 6, 11, 16, 18 for 2-dose girls compared with 3-dose girls and 3-dose women.

Co-primary endpoints are the antibody levels measured by MERCK using competitive Luminex immunoassay (cLIA) at 7 and 30 days post-booster dose of HPV vaccine.

Co-secondary endpoints are the antibody levels measured by MERCK using total IgG Luminex immunoassay (TIgG) at 7 and 30 days post-booster dose of HPV vaccine. Antibody levels measured by cLIA and TIgG at baseline will also be compared in the three groups.

Statistical Analysis:

Participation and demographic/clinical Characteristics:

Descriptive analysis (i.e. frequency and/or percentage) will be used to summarize study population, including enrollment, withdraw, loss to follow-up, eligibility (Figure 1); and demographic/clinical characteristics including age, baseline health and immunization history (Table 1).

Evaluation of anti-HPV antibody levels:

Merck employed cLIA and TIgG to measure neutralizing and total IgG antibody response to 9-valent HPV vaccine. To assess anti-HPV antibody levels, we will use the results of cLIA and total IgG at baseline (visit 1), and at 7 days (visit 2) and 30 days (visit 3) post-booster dose of HPV vaccine. GMTs of anti-HPV 6, 11, 16, and 18 antibodies will be computed for each group at the three time points. To allow GMT calculation, samples with undetectable anti-HPV will be assigned the half of the lowest detectable value (*cLIA: HPV6, 2 mMU/ml; HPV11, 3 mMU/ml; HPV16, 5 mMU/ml; HPV18, 10 mMU/ml. TIgG:*

HPV6, 2 mMU/ml; HPV11, 2 mMU/ml; HPV16, 4 mMU/ml; HPV18, 3 mMU/ml). 95% confidence intervals (CIs) of GMT will be calculated by back transformation of the 95% CI for the log-transformed GMT (Table 2-3).

Non-inferiority testing:

To test non-inferiority, t-test will be applied to assess GMT ratios in different groups (2-dose girls vs. 3-dose girls; 2-dose girls vs. 3-dose women; 3-dose girls vs. 3-dose women) at 7 and 30 days post-booster dose of HPV vaccine. Non-inferiority will be declared if the lower bound of the 95% confidence interval (95% CI) of GMT ratios is greater than 0.5 (Table 2-3).

Evaluation of seropositivity:

Seropositivity will be examined for the three groups at baseline, and at 7 and 30 days post-booster dose of HPV vaccine. Positivity thresholds for cLIA are 20 mMU/mL, 16mMU/mL, 20 mMU/mL, and 24 mMU/mL for anti-HPV 6, 11, 16, and 18, respectively; whereas total IgG 15 mMU/mL, 15mMU/mL, 7 mMU/mL, and 10 mMU/mL respectively. A participant is considered to be seropositive if the respective anti-HPV antibody level exceeds or equal to these threshold (Table 4-5). Chi-squared test will be used to compare seropositivity rate in different groups (2-dose girls vs. 3-dose girls; 2-dose girls vs. 3-dose women; 3-dose girls vs. 3-dose women) at baseline, and at 7 and 30 days post-booster dose of HPV vaccine. A margin of 10% of difference in seropositivity rate will be used for non-inferiority testing.

Assessment of difference in antibody response over time:

Difference in antibody response over time will be assessed based on log-transformed scale of GMT values at different time points (baseline vs. 7 days; baseline vs. 30 days). Paired t-test will be used to test the difference in each group over time. Two-sample t-test will be used to test difference over time between groups (2-dose girls vs. 3-dose girls; 2-dose girls vs. 3-dose women; 3-dose girls vs. 3-dose women). All of the t-test will be two-sided, at a significant level of 0.05 (Table 6-7).

Figures and Tables:

Figure 1. Study participation consort diagram to summarize enrollment, withdraw, loss of follow-up, eligibility…

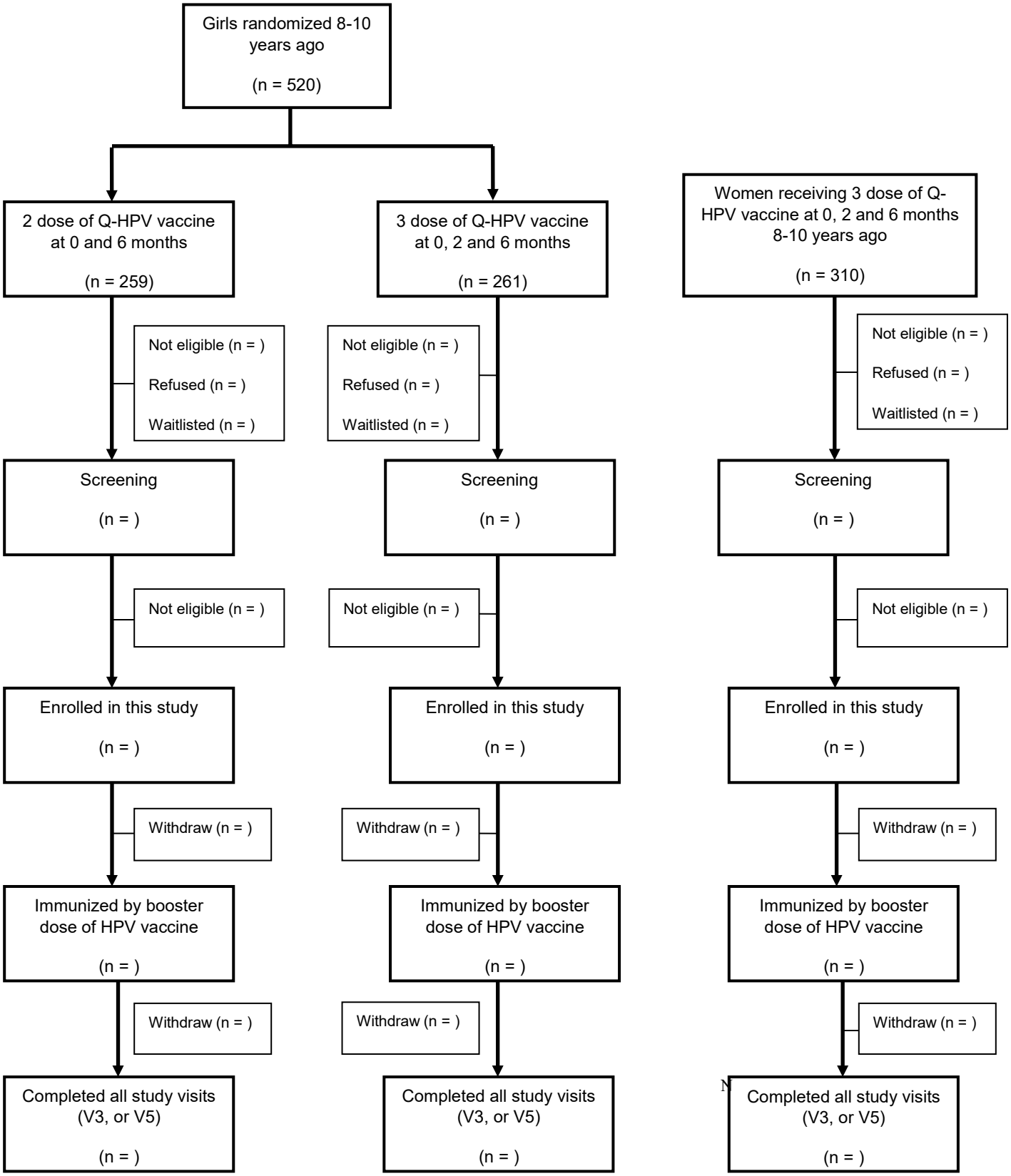

Table 1. Demographic/clinical characteristics of the study population

Characteristics	Group 1 (2-dose girls)		Group 2 (3-dose girls)		Group 3 (3-dose women)	
	N	%	N	%	N	%
Age at enrollment, mean (SD), year **Age at first dose of Q-HPV vaccine**, mean (SD), year						
Weight, mean (SD), kg						
BMI, mean (SD)						
Ethnicity White Chinese Other						
Health history						
Immunization history						

G*roup 1*: *girls who received 2 doses of Q-HPV vaccine at 0, 6 month schedule 8-10 years ago when they were between 9-13 years of age at the time of the first dose*

Group2: *girls who received 3 doses of Q-HPV vaccine at 0, 2, 6 month schedule 8-10 years ago when they were between 9-13 years of age at the time of the first dose*

Group 3: *young women who received 3 doses of Q-HPV vaccine at 0, 2, 6 month schedule 8-10 years ago when they were between 16-26 years of age at the time of the first dose*)

Table 2. Summary of anti-HPV GMTs at baseline, and 7 and 30 days post-booster dose (cLIA)

Antibodies (cLIA)	Group 1 (2-dose girls)		Group 2 (3-dose girls)		Group 3 (3-dose women)		GMT ratio (95% CI)		
	n	GMT (95% CI)	n	GMT (95% CI)	n	GMT (95% CI)	2-dose girl/3-dose girl	2-dose girl/3-dose women	3-dose girl/3-dose women
Baseline									
HPV 6									
HPV 11									
HPV 16									
HPV 18									
At 7 days post-booster dose									
HPV 6									
HPV 11									
HPV 16									

HPV 18									
At 30 days post-booster dose									
HPV 6									
HPV 11									
HPV 16									
HPV 18									

Table 3. Summary of anti-HPV GMTs at baseline, and 7 and 30 days post-booster dose (total IgG)

Antibodies (IGG)	Group 1 (2-dose girls)		Group 2 (3-dose girls)		Group 3 (3-dose women)		GMT ratio (95% CI)		
	n	GMT (95% CI)	n	GMT (95% CI)	n	GMT (95% CI)	2-dose girl/3-dose girl	2-dose girl/3-dose women	3-dose girl/3-dose women
Baseline									
HPV 6									
HPV 11									
HPV 16									
HPV 18									
At 7 days post-booster dose									
HPV 6									
HPV 11									
HPV 16									
HPV 18									
At 30 days post-booster dose									
HPV 6									
HPV 11									
HPV 16									
HPV 18									

Table 4. Summary of HPV seropositivity at baseline, and at 7 and 30 days post-booster dose (cLIA)

Antibodies (cLIA)	Group 1 (2-dose girls)		Group 2 (3-dose girls)		Group 3 (3-dose women)	
	n	% seropositive (95% CI)	n	% seropositive (95% CI)	n	% seropositive (95% CI)
Baseline						
HPV 6						
HPV 11						
HPV 16						

HPV 18						
At 7 days post-booster dose						
HPV 6						
HPV 11						
HPV 16						
HPV 18						
At 30 days post-booster dose						
HPV 6						
HPV 11						
HPV 16						
HPV 18						

Table 5. Summary of HPV seropositivity at baseline, and 7 and 30 days post-booster dose (total IGG)

Antibodies (IGG)	Group 1 (2-dose girls)		Group 2 (3-dose girls)		Group 3 (3-dose women)	
	n	% seropositive (95% CI)	n	% seropositive (95% CI)	n	% seropositive (95% CI)
Baseline						
HPV 6						
HPV 11						
HPV 16						
HPV 18						
At 7 days post-booster dose						
HPV 6						
HPV 11						
HPV 16						
HPV 18						
At 30 days post-booster dose						

HPV 6						
HPV 11						
HPV 16						
HPV 18						

Table 6. Difference in anti- HPV GMTs between baseline vs. at 7 and 30 days post-booster dose of HPV vaccine (cLIA)

Antibodies (cLIA)	Group 1 (2-dose girls)		Group 2 (3-dose girls)		Group 3 (3-dose women)		Group 1 vs 2	Group 1 vs 3	Group 1 vs 3
	Log Difference	P Value	Log Difference	P value	Log Difference	P value	P-value for difference between differences for group 1 vs 2	P-value for difference between differences for group vs 3	P-value for difference between differences for group 1 vs 3
Baseline vs. 7 days post-booster dose of HPV vaccine									
HPV 6									
HPV 11									
HPV 16									
HPV 18									
Baseline vs. 30 days post-booster dose of HPV vaccine									
HPV 6									
HPV 11									
HPV 16									
HPV 18									

Table 7. Difference in anti- HPV GMTs between baseline vs. at 7 and 30 days post-booster dose of HPV vaccine (total IgG)

Antibodies (IGG)	Group 1 (2-dose girls)		Group 2 (3-dose girls)		Group 3 (3-dose women)		Group 1 vs 2	Group 1 vs 3	Group 1 vs 3
	Log Difference	P Value	Log Difference	P value	Log Difference	P value	P-value for difference between differences	P-value for difference between differences for group	P-value for difference between differences

							for group 1 vs 2	vs 3	for group 1 vs 3
Baseline vs. 7 days post-booster dose of HPV vaccine									
HPV 6									
HPV 11									
HPV 16									
HPV 18									
Baseline vs. 30 days post-booster dose of HPV vaccine									
HPV 6									
HPV 11									
HPV 16									
HPV 18									

Tables for uploading results to ClinicalTrials.gov, as required.