Statistical Analysis Plan to Address the Primary and Secondary Objectives for the Trial Entitled

"Non-inferiority trial comparing immunogenicity from 1-dose of bivalent HPV vaccine in girls to 3-doses of quadrivalent vaccine in women: The PRIMAVERA-ESCUDDO Trial ('Puente de Respuesta Inmunológica para Mejorar el Acceso a Vacunas y ERrAdicar el cancer')**"**

(NCI Protocol 19-C-009**)**

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1. Introduction

This document describes the pre-planned analysis for the trial entitled "**Non-inferiority trial comparing immunogenicity from 1-dose of bivalent HPV vaccine in girls to 3-doses of quadrivalent vaccine in women"**.

2. Objectives

2a. Primary Objective

Primary Objective (PO): To demonstrate that the immunogenicity (as determined by ELISA) of a single dose of Cervarix in 9-14 year old girls is non-inferior to the immunogenicity of three doses of Gardasil, administered at 0, 2, and 6 months, in 18-25 year old women 36 months after initial vaccination, with an interim analysis at 24 months after initial vaccination.

Either of following criteria will qualify as non-inferiority:

- The interim analysis will assess non-inferiority at 24 months. Non-inferiority will be demonstrated if the lower limits for the two-sided 99% confidence intervals of the GMT ratios (1-Dose Cervarix divided by 3-Doses of Gardasil at 24 months) for both HPV-16 and HPV-18 antibody levels exceed 0.67.
- The primary analysis will assess non-inferiority at 36 months. Non-inferiority will be demonstrated if the lower limits for the two-sided 96% confidence intervals of the GMT ratios (1-Dose Cervarix divided by 3-Doses of Gardasil at 36 months) for both HPV-16 and HPV-18 antibody levels exceed 0.67.

2b. Secondary Objectives

Secondary Objective 1 (SO1): To compare the distribution of both HPV-16 and HPV-18 antibodies levels, assessed at 24 and 36 months after initial vaccination, following a single dose of Cervarix in 9-14 year old girls and following three doses of Gardasil, administered at 0, 2, and 6 months, in 18-25 year old women.

Secondary Objective 2 (SO2): To compare rates of seroconversion based on both HPV-16 and HPV-18 antibody levels, assessed at 24 and 36 months after initial vaccination, following a single dose of Cervarix in 9-14 year old girls and following three doses of Gardasil, administered at 0, 2, and 6 months, in 18-25 year old women.

Secondary Objective 3 (SO3): To compare GMTs, distributions, and seroconversion rates for HPV-16 and HPV-18 antibodies, assessed at 24 and 36 months after initial vaccination, following a single dose of Cervarix in 11-14 year old girls and following three doses of Gardasil, administered at 0, 2, and 6 months, in 18-25 year old women.

Secondary Objective 4 (SO4): To compare GMTs, distributions, and seroconversion rates for HPV-16 and HPV-18 antibodies, assessed at 1-month and 1-year after vaccination, following a single dose of Cervarix in 9-10 year old girls and following a single dose of Cervarix in 11-14 year old girls.

Secondary Objective 5 (SO5): To evaluate whether baseline variables (i.e. age, enrollment date, district) are associated with GMTs, distributions, and seroconversion rates for HPV-16 and HPV-18 antibodies, assessed at 24 and 36 months after initial vaccination.

We note that SO3 and SO4 are, in part, included in this study because of Costa Rica's national vaccination program that aimed to vaccinate 10-year-old girls starting in 2019.

3. Analytical Cohorts

3a. Primary Analytical Cohort

The According to Protocol (ATP) cohort for a specified HPV type (i.e. HPV 16 or HPV 18) and timing (i.e. 24-month or 36-month) are the participants who satisfy the following set of requirements:

- Received the correct number of doses within the predefined vaccination windows. Denoting T_1 as the time of the first vaccination, the window for the second vaccination is between T_1+30 days and T_1+90 days. Denoting T_2 as the time of the second vaccination, the window for the third vaccination is between T_2+90 days and T_1+360 days.

- Not seropositive at baseline for the specified HPV type.

- Blood collected within the predefined follow-up window. Denoting T_1 as the time of the first vaccination, the window for the 24-month visit is between T_1+700 days and T_1+912 days and the window for the 36-month window is T_1+1065 days and T_1+1278 days.

- No HPV vaccination outside of the study prior to blood collection. The participant did not report receiving an HPV vaccine and, among those tested, did not have HPV6 and HPV11 vaccine-level antibodies.

Note, the ATP cohort for a given analysis depends on both the timing of the analysis and the specified HPV type.

3b. Secondary/Exploratory Analytical Cohorts

We will also consider the: (i) modified Intention-to-Treat (ITT) cohort, including all participants receiving the correct number of doses and with blood collected during the predefined follow-up window; (ii) Seropositive-ATP (SP-ATP) cohort that includes participants who are seropositive at baseline, but otherwise satisfy the ATP requirements; and (iii) Serocombined-ATP (SC-ATP) cohort that combines the ATP and SP-ATP cohorts. Furthermore, for SO4, we will consider all

girls who are not seropositive at baseline, had blood collected between T_1 + 14 and T_1 + 90 days for the 1 month analysis and between T_1 + 274 and T_1 + 547 days for the 1 year analysis, and did not receive an HPV vaccination outside of the study prior to their blood collection. Additionally, we will test for HPV6/HPV11 antibodies in a subset of participants who are at-risk for external HPV vaccination or show serologic evidence of additional HPV vaccine doses.

4. Endpoints

The four endpoints are HPV-16 and HPV-18 specific antibody results obtained from blood specimens (serum) collected at the 24- (interim) and 36-(primary) month visit (i.e. 4 endpoints = 2 HPV types x 2 time-points). All antibody levels are measured by HPV type-specific ELISA. Two additional endpoints, HPV-16 and HPV-18 antibody levels measured from serum collected at the 1-month visit and the 1-year visit, will also be included to achieve Secondary Objective 4 (SO4).

5. Implementation of Analysis

The final 24- and 36-months analyses will be performed once the corresponding follow-up window for the last enrolled participant closes

6. Controlling the Type-I error rate

The analysis defined in the following section ensures that under the null hypothesis of inferiority, the probability of declaring non-inferiority (i.e. type I error rate) is less than 0.025 for HPV-16 and HPV-18 endpoints, respectively.

7. Statistical Analyses for Immunogenicity

7a. Methods for The Primary Objective

We will estimate the GMT ratios for HPV-16 and HPV-18 at 24 and 36 months in the corresponding ATP cohort.

We define $R^H(t) = GMT_1^H(t)/GMT_3^H(t)$ to be the GMT ratio for HPV-H (i.e. HPV-16 or HPV-18) at time point t (i.e. 24 or 36 months) when comparing antibody levels in 9-14 year old girls receiving 1 dose of Cervarix to levels in 18-25 year old women receiving 3 doses of Gardasil.

We will estimate $R^H(t)$ by fitting a generalized estimating equation (GEE) model where the dependent variable is the log-antibody level and the independent variable is study-group. Denoting the estimated coefficient for study-group by $\hat{\beta}^H(t)$ and the corresponding robust standard error by $\hat{\sigma}^H(t)$, we define our estimate by $\hat{R}^H(t) = \exp(\hat{\beta}^H(t))$ and define the two-sided (1-α)% confidence interval (CI), $(\hat{R}^H_{\alpha/2}(t), \hat{R}^H_{1-\alpha/2}(t))$, by $\exp(\hat{\beta}^H(t) \pm z_{1-\alpha/2}\hat{\sigma}^H(t))$, where $z_{1-\alpha/2}$ is the 1-α/2 quantile of a normal distribution. In the model, we will group girls into clusters of full siblings and use an exchangeable correlation matrix.

We will declare non-inferiority if $\min\left(\hat{R}^{16}_{0.005}(24), \hat{R}^{18}_{0.005}(24)\right) > 0.67$ or, upon failure of that criterion, $\min\left(\hat{R}^{16}_{0.02}(36), \hat{R}^{18}_{0.02}(36)\right) > 0.67$. Note, under the null hypothesis that both $\min\left(R^{16}(24), R^{18}(24)\right) < 0.67$ and $\min\left(R^{16}(36), R^{18}(36)\right) < 0.67$, the probability of declaring non-inferiority or the Type I error rate is less than 0.025.

7b. Methods for The Secondary Objectives

Secondary Objective 1 (SO1): For each combination of HPV-type, time-point, and study group, we will describe the distribution of antibody levels by showing the reverse cumulative distributions (i.e. 1-cumulative distribution function) in the ATP cohort.

Secondary Objective 2 (SO2): For each combination of HPV-type, time-point, and study group, we will report the proportion of the ATP cohort who seroconvert (i.e. achieve HPV-16 or HPV-18 antibody levels greater than 1.41 international units (IU)/mL or 1.05 IU/mL, respectively) and exact 95% confidence intervals. For each HPV-type and time-point, we will assess whether seroconversion rates differ between study groups using fisher's exact test.

Secondary Objective 3 (SO3): We will repeat the analyses described for the primary objective and secondary objectives 1 and 2 restricted to 11-14-year-old girls. We will also perform a direct comparison of the antibody levels in 9-10 and 11-14 year old girls (e.g. compare GMT in GEE w/ age group as the independent variable, compare seroconversion rates by fisher's exact test).

Secondary Objective 4 (SO4): We will repeat the analyses described for the primary objective and secondary objectives 1 and 2 to compare antibody levels at 1-month and 1-year post-vaccination in girls 9-10 years old girls and in girls 11-14 years old.

Secondary Objective 5 (SO5): We will consider the following baseline covariates: age, enrollment date, and district. Within each study-group and at each time point, we will report the GMTs and their 95% CIs for HPV-16 and HPV-18 antibody levels in subgroups defined by these baseline covariates. Moreover, we will use GEEs to assesses the association between GMTs and these baseline covariates and show both reverse cumulative distributions and seroconversion rates by subgroup.

7c. Methods for Sensitivity and Exploratory Analyses

We will repeat the methods for the primary and secondary objectives using the ITT, SP-ATP, and SC-ATP cohorts instead of the ATP cohort.

We will repeat the methods for the primary and secondary objectives after removing potential outliers (e.g. participants potentially exposed to a vaccination outside of study) where the antibody level is 2xIQR above the 75th percentile.

We will assess whether antibody levels are changing over time. For each combination of study group and HPV type, we will compare GMTs at 24 and 36 months.

8. Statistical Analyses for Evaluating ELISA assays And Comparability with SEAP-NA

HPV binding is measured by ELISA. The quantity of HPV16 and 18 IgG antibody response measured by VLP ELISA will be utilized to address immunogenicity endpoints. To further define the primary analytic cohort, HPV6 and HPV11 antibodies will be measured by VLP ELISA in a subset of participants to confirm, beyond self-report, that the according to protocol analytic cohort includes only girls who did not receive external HPV vaccination through the national program, which uses quadrivalent vaccine (HPV 6, 11, 16, 18). This will assure that girls in the single-dose HPV vaccination arm did not get additional doses.

HPV neutralization is measured by SEAP-NA. Antibodies levels measured by ELISA will be compared to neutralizing antibody levels measured by the pseudovirion-based neutralization assay, SEAP-NA. This nested study will select 24- month serum samples from a random set of 100 baseline seronegative 18-25 year old women and a random set of 100 baseline seronegative 9-14 year old girls, and then evaluate the HPV-16 and HPV-18 antibody levels in each of those 200 samples using both ELISA and SEAP-NA. For each HPV type, we will perform a Deming regression comparing log(ELISA) levels with log(SEAP) levels, where log(ELISA) is the dependent variable and log(SEAP) is the independent variable. We will confirm that ELISA is an acceptable assay for the primary endpoint if the 95% confidence intervals of the regression slope excludes $1-\Delta$ and $1+\Delta$ for both HPV types, with $\Delta = 0.3$. If that criterion is not achieved, then we will initiate a discussion with relevant parties to assess acceptability of the assay.