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TITLE:
A Registry-Based Extension of Protocol V503-001 in Countries with Centralized Cervical Cancer Screening Infrastructures to Evaluate the Long-Term Effectiveness, Immunogenicity, and Safety of Multivalent Human Papillomavirus (HPV) L1 Virus-Like Particle (VLP) Vaccine as Administered to 16- to 26- Year- Old Women.

INVESTIGATOR:

PRIMARY:

CLINICAL PHASE: III

US IND NUMBER: 13447

SITE:

INSTITUTIONAL REVIEW BOARD/ETHICS REVIEW COMMITTEE:

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SUMMARY OF CHANGES

PRIMARY REASON(S) FOR THIS AMENDMENT

Section Num-ber(s)	Section Title(s)	Description of Change(s)
		The primary purpose of the Protocol 021-01 amendment is to simplify the conduct of the study without altering the overall goals of the study. Specifically the following changes are being made: • Registry searches will be conducted for all enrolled subjects as initially planned. However, only those Subjects who received the 9-valent HPV L1 VLP (9vHPV) vaccine in the base study, Cohort 1, will be followed for the effectiveness and immunogenicity study objectives. Cohort 2 subjects, those who have either received GARDASIL™ only and/or subjects who received GARDASIL™ followed by the 9vHPV vaccine in the base study, may be followed for exploratory analyses. • When this protocol was originally written, it was anticipated that subjects would exit the V503-001 base study over a period of approximately 1.5 to 2 years, and for this reason, the protocol was designed with 6 interim analyses. However, all of the Cohort 1 Subjects exited the base study at approximately the same time. Therefore, the number of Interim Periods has been reduced to a total of 5. The total duration of follow-up is unchanged; all subjects will be followed for a total of 10 years. • In the original protocol, serum samples were to be collected from all subjects at Year 5 and Year 10. The protocol is revised so that serum will be collected from 20% of subjects who received the 9vHPV vaccine in the base study (i.e., the same subset of subjects who were evaluated for antibody persistence in the base study). Moreover, serum samples for the Year 5 and 10 immunogenicity testing will be collected only from the subjects in Denmark. Denmark has approximately 83% of the subjects in Protocol 021-01 which will provide a sufficient sample for the statistical analysis.

Section Num-ber(s)	Section Title(s)	Description of Change(s)
		• Registry searches will be conducted in all 3 countries as planned for every 2 year Interim Period. However, the collection and analysis of tissue specimens will be rolled out based upon the expected number of cases. The tissue specimens from Denmark, (represents ~83% of the total enrollment) will be collected and analyzed from the beginning of the study. In Norway (represents ~14% of the total enrollment) the tissue specimens will be collected and analyzed beginning with the 2nd Interim Report. The tissue specimens from Sweden (represents ~3% of the total enrollment) will only be collected and analyzed at the end of the study for the Final Report.

ADDITIONAL CHANGE(S) FOR THIS AMENDMENT

Section Num-ber(s)	Section Title(s)	Description of Change(s)	Rationale
1.5	SAMPLE	Clarified Cohort 2 may be followed for exploratory analyses.	Data from the Cohort 2 subjects may be difficult to interpret because subject HPV status prior to 9VHPV vaccine administration is not completely known.
1.7	STUDY FLOW CHART	Inserted Country Specific Study Flow Charts.	To add clarity since the timing for the study activities is different in each country.
2.4.1	SUMMARY OF STUDY DESIGN	Clarified the effectiveness and safety analyses that will occur.	Collection and analysis of tissue specimens for the effectiveness analysis will be based on the expected case number which is related to the number of subjects in each country.

Section Num-ber(s)	Section Title(s)	Description of Change(s)	Rationale
2.4.1.2	Active Follow-Up	Clarified that the subjects who will be providing serum for immunogenicity testing will be a subset of subjects from Denmark.	Approximately 20% of the subjects from the base study V503-001 participated in an antibody persistence sub-study

1. SUMMARY

1.1 TITLE

A Registry-Based Extension of Protocol V503-001 in Countries with Centralized Cervical Cancer Screening Infrastructures to Evaluate the Long-Term Effectiveness, Immunogenicity, and Safety of Multivalent Human Papillomavirus (HPV) L1 Virus-Like Particle (VLP) Vaccine as Administered to 16- to 26-Year-Old Women.

1.2 INDICATION

Evaluation of the long-term effectiveness, immunogenicity, and safety of the 9-valent HPV L1 VLP vaccine.

1.3 SUMMARY OF RATIONALE

V503 is a prophylactic 9-valent HPV (Types 6, 11, 16, 18, 31, 33, 45, 52, and 58) L1 VLP vaccine[1] that is comprised of VLPs of the 4 HPV types (Type 6, 11, 16, and 18) represented in GARDASIL™[2], plus the VLPs of 5 additional oncogenic HPV types (Type 31, 33, 45, 52, and 58). This vaccine offers the potential of significant prophylactic cancer coverage in addition to that already provided by GARDASIL™, with an increase in overall cervical cancer coverage from approximately 70% to 90%. This is in addition to the potential of coverage for genital warts provided by VLPs of HPV Types 6 and 11.

The Protocol V503-001 base study is a randomized, worldwide study to evaluate dose-ranging, safety/tolerability, immunogenicity and efficacy of V503 in young women, 16 to 26 years of age. Approximately 14,000 subjects were enrolled in the study for efficacy evaluation and randomized to V503 or the active control, GARDASIL™. Subjects were followed in the V503-001 base study for up to 54 months.

Protocol V503-021 is a study extension of the V503-001 base study to evaluate the safety, immunogenicity, and long-term effectiveness of V503 in preventing cervical, vulvar, and vaginal cancers and related precancers caused by the vaccine HPV types (6, 11, 16, 18, 31, 33, 45, 52, and 58). This protocol number, 021, differs from the base protocol number, 001, to allow for the establishment of a new, separate clinical electronic database by the SPONSOR.

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[1] The 9-valent HPV (Types 6, 11, 16, 18, 31, 33, 45, 52, and 58) L1 VLP vaccine will hereafter be referred to as 9vHPV vaccine in this protocol

[2] GARDASIL [Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine] is a registered trademark of Merck & Co., Inc., Whitehouse Station, New Jersey, U.S.A. GARDASIL is also known as SILGARD in some countries. SILGARD is a trademark of Merck & Co., Inc., Whitehouse Station, New Jersey, U.S.A.

1.4 SUMMARY OF STUDY DESIGN

This Long-Term Follow-Up (LTFU) V503-021 study is an extension of the Protocol V503-001 base study in the Nordic Region countries of Denmark, Norway and Sweden.

In the Nordic region, 4453 subjects were enrolled into the V503-001 base study, as shown in Table 1-1

Table 1-1

Subject Enrollment In Base Study Per Country

Country	Total Number of Subjects Enrolled into the V503-001 Base Study
Denmark	3689
Norway	637
Sweden	127
Total	4453

With more than 90% subject retention in the Nordic countries, most of the subjects from the base study will be followed in the LTFU study through the highly efficient screening and surveillance system that exists in the Nordic countries. Currently, the national centralized cervical cancer screening programs in Denmark, Norway and Sweden recommend that women have Papanicolaou (Pap) tests every 3 years. In the coming years, this recommendation is expected to be updated to also include other HPV tests for screening, triage, and diagnosis in these same countries.

The Investigators from each of these 3 countries will lead the National Registry Study Centers (NRSCs), which will obtain Pap tests and histopathology results from biopsies and definitive therapy specimens, as well as results for other HPV tests for screening, triage and diagnosis, which are collected by subject Personal Identification Number (PIN) in national data systems called registries. The registries routinely obtain biopsy slides and tissue blocks for research analyses. Therefore, the registry data systems can be used to investigate the effectiveness of the 9vHPV vaccine after the Protocol V503-001 base efficacy study ends.

The LTFU study will start for each subject upon completion of her last Protocol V503-001 base study visit. Since women residing in the Nordic region were instructed not to participate in their national cervical screening program while they were enrolled in the Protocol V503-001 base study, the NRSCs will ensure that subjects are informed to resume participation in the national cervical cancer screening program. In addition, the NRSCs will:

1. Search the cervical cancer screening registries periodically to identify Pap testing, genital tract biopsy, or definitive therapy results, as well as results for other HPV tests for screening, triage, and diagnosis,

2. Provide the local Pap test, biopsy, and definitive therapy diagnoses, as well as, any results available for other HPV tests for screening, triage, and diagnosis, to the SPONSOR.

3. Obtain biopsy and definitive therapy slides and blocks from the local pathology laboratories and send them to the central laboratory.

4. Search health-related registries of hospitalizations to find safety events of interest.

5. Coordinate serum collection for HPV serological testing at years 5 and 10 of the LTFU study.

When NRSCs provides the tissue blocks, the pathology laboratory will:

1. Cut tissue for Thinsection Polymerase Chain Reaction (PCR) testing for 14 HPV types (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59)

2. Create new Hemotoxylin & Eosin (H & E) slides for diagnosis by the Nordic Pathology Panel (NPP).

1.5 SAMPLE

The Protocol V503-001 base study includes 4453 women in Denmark, Norway, and Sweden. These subjects were randomized in a 1:1 ratio and received either 9vHPV vaccine or GARDASIL™ during the base study. More than 90% of the subjects from these Nordic Countries continue to participate in the base study and are expected to enter into the LTFU study.

Cohort 1: Approximately 50% of the subjects received 9vHPV vaccine in the Protocol V503-001 base study and will each contribute approximately 14 years of follow-up after vaccination (Approximately 4 years within Protocol V503-001 and 10 years within the LTFU study).

Cohort 2: Approximately 50% of the subjects received GARDASIL™ in the Protocol V503-001 base study and were offered the 9vHPV vaccine at the conclusion of the base study, in the context of a study extension (Protocol V503-001-04). Participation in Protocol V503-001-04 is on a voluntary basis. These subjects who have either received GARDASIL™ only (i.e., they did not participate in Protocol V503-001-04) and the subjects who received GARDASIL™ followed by the 9vHPVvaccine in Protocol V503-001-04, together, may be assessed in Protocol V503-021-01 in exploratory analyses.

All subjects who consented to participate in the LTFU study will be followed utilizing the Allocation Number (AN)–to- PIN mapping which was established at each NRSC.

1.6 DOSAGE/DOSAGE FORM, ROUTE, AND DOSE REGIMEN

No vaccinations will occur within the context of the LTFU study.

1.7 STUDY FLOW CHART

Denmark

Key Scheduled Tests and Events	Within Protocol V503-001 (Base Study)	Time (years) following Last Patient Out (LPO) of V503-001 Base Study[1]										
		2.0	3.0	4.0	5.0‡	6.0	7.0	8.0	9.0	10.0‡		
Obtain Informed Consent[2]	x											
Search registries for Pap smear, other HPV tests for screening, triage or diagnosis, biopsy, and definitive therapy procedures[3]		Ongoing →										
Obtain slides for adjudication[3]		Ongoing →										
Obtain blocks for sectioning new slides (for adjudication process) and thinsections (For PCR-testing)[3]		Ongoing →										
Search registries for new medical history[4]		Ongoing →										
Obtain serum for HPV antibody measurements including cLIA and IgG (including Retention Serum)[5,6]					x					x		
Analysis of search results for effectiveness objectives		x		x		x		x		x		

[1] For the analyses, a subject's start date in Protocol V503-021 is their exit date from the Protocol V503-001 base study.
[2] Informed Consent for Protocol V503-021 will be obtained within the base study Protocol V503-001 per local requirements.
[3] These activities are conducted for Cohort 1 only.
[4] Search of registries for new medical history will be for both Cohorts 1 and 2.
[5] Serum specimens will be collected from a subset of subjects (see Administrative Binder) where cLIA and IgG testing will be performed. Serum must be shipped as specified by the SPONSOR/Central Laboratory. The Retention Serum vial must remain at the site until the SPONSOR notifies the study site to ship the samples.
[6] There are no protocol-defined windows for the serum collection visit. Samples from subjects will be collected during two blood collection campaigns: one approximately 5 years and one approximately 10 years following the start of the LTFU study.

Norway

Key Scheduled Tests and Events	Within Protocol V503-001 (Base Study)	Time (years) following Last Patient Out (LPO) of V503-001 Base Study[1]										
		2.0	3.0	4.0	5.0‡	6.0	7.0	8.0	9.0	10.0‡		
Obtain Informed Consent[2]	x											
Search registries for Pap smear, other HPV tests for screening, triage or diagnosis, biopsy, and definitive therapy procedures[3]		Ongoing →										
Obtain and provide slides to the central laboratory for adjudication[3]				Ongoing →								
Obtain and provide blocks for adjudication process and thinsection PCR testing[3]				Ongoing →								
Search registries for new medical history[4]		Ongoing →										
Analysis of search results for effectiveness objectives		x[5]		x		x		x		x		

[1] For the analyses, a subject's start date in Protocol V503-021 is their exit date from the Protocol V503-001 base study.
[2] Informed Consent for Protocol V503-021 will be obtained within the base study Protocol V503-001 per local reqiurements.
[3] These activities are conducted for Cohort 1 only.
[4] Search of registries for new medical history will be for both Cohorts 1 and 2.
[5] The first interim analysis will be limited to the data from the registry searches and not include processing and testing of tissue samples. ¶

Sweden

Key Scheduled Tests and Events	Within Protocol V503-001 (Base Study)	Time (years) following Last Patient Out (LPO) of V503-001 Base Study[1]										
		2.0	3.0	4.0	5.0‡	6.0	7.0	8.0	9.0	10.0‡		
Obtain Informed Consent[2]	x											
Search registries for Pap smear, other HPV tests for screening, triage or diagnosis, biopsy, and definitive therapy procedures[3]		Ongoing →										
Obtain and provide slides to the central laboratory for adjudication[3]										x		
Obtain and provide blocks for adjudication process and thinsection PCR testing[3]										x		
Search registries for new medical history[4]		Ongoing →										
Analysis of search results for effectiveness objectives		x[5]		X[5]		X[5]		X[5]		x		

[1] For the analyses, a subject's start date in Protocol V503-021 is their exit date from the Protocol V503-001 base study.
[2] Informed Consent for Protocol V503-021 will be obtained within the base study Protocol V503-001.
[3] These activities are conducted for Cohort 1 only.
[4] Search of registries for new medical history will be for both Cohorts 1 and 2.
[5] The first four interim analyses will be limited to the data from the registry searches and not include processing and testing of tissue samples. ¶

2. CORE PROTOCOL

2.1 OBJECTIVES AND HYPOTHESES

The following objectives (Primary, Secondary, and Exploratory) will be conducted in Cohort 1.

2.1.1 Primary

Objective: To assess the long-term effectiveness of the 9vHPV vaccine by monitoring the combined incidence of Cervical Intraepithelial Neoplasia (CIN) 2, CIN 3, Adenocarcinoma In Situ (AIS) and cervical cancer related to HPV 16, 18, 31, 33, 45, 52, and 58 in women from Protocol V503-001 in the Nordic region vaccinated with the 9vHPV vaccine

Hypothesis: 9vHPVvaccine will remain effective for at least 14 years after the start of vaccination. (*Cases of HPV 16/18/31/33/45/52/58-related CIN2, CIN3, AIS, and cervical cancer will be monitored using a control chart where a signal indicates that the vaccine effectiveness has decreased to less than 90%.*)

2.1.2 Secondary

(1) **Objective:** To evaluate the risk for long-term type replacement by monitoring the combined incidence of CIN 2, CIN 3, AIS, and cervical cancer related to HPV 35, 39, 51, 56 and 59 in women from Protocol V503-001 in the Nordic region vaccinated with V503.

(2) **Objective:** To estimate long-term effectiveness of 9vHPVvaccine against CIN 1, CIN 2, and CIN 3, AIS, cervical cancer, vulvar cancer (in situ or invasive), or vaginal cancer (in situ or invasive) related to HPV 6, 11,16, 18, 31, 33, 45, 52, and/or 58.

(3) **Objective:** To evaluate the long-term HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 antibody responses generated by the 9vHPVvaccine.

2.1.3 Exploratory

(1) **Objective:** To describe the incidence of CIN grades 2 or 3, AIS, or cervical cancer, as defined in the primary endpoint, but irrespective of HPV-type relatedness.

(2) **Objective:** To describe the incidence of CIN 1, CIN 2, and CIN 3, AIS, cervical cancer, vulvar cancer (in situ or invasive), or vaginal cancer (in situ or invasive), as defined in the secondary endpoint, but irrespective of HPV-type relatedness.

(3) **Objective:** To describe the incidence of Pap test abnormalities.

(4) **Objective:** To explore the relationship between antibody level and disease breakthrough, data permitting.

2.2 SUBJECT/PATIENT INCLUSION CRITERIA

1. Subject was randomized into Protocol V503-001 from Denmark, Norway, or Sweden and participated in the study by either receiving the selected 9vHPVvaccine dose formulation (30/40/60/40/20/20/20/20/20 mcg each of HPV Types 6/11/16/18/31/33/45/52/58) or GARDASIL™.

2. Subject agrees to allow passive follow-up, analysis of biopsy specimens, future contact from the NRSC, and serum collection for this LTFU study and has provided written consent as needed per local requirements.

2.3 SUBJECT/PATIENT EXCLUSION CRITERIA

1. There are no exclusion criteria.

2.4 STUDY DESIGN AND DURATION

2.4.1 Summary of Study Design

Protocol V503-001 is an international, multicenter, efficacy, immunogenicity, and safety study of the 9vHPVvaccine. Approximately 14,000 young women, 16 to 26 years of age, were enrolled in the study and were administered a 3-dose regimen of 9vHPVvaccine or GARDASIL™ at Day 1, Month 2, and Month 6. Protocol V503-001 was designed for a follow-up of up to 54 months (or 48 months postvaccination).

The LTFU study, Protocol V503-021, is designed to evaluate longer-term effectiveness, immunogenicity, and safety of the 9vHPVvaccine and GARDASIL™ for at least 10 years following completion of the base study of Protocol V503-001. The LTFU study will be conducted in subjects in the Nordic region who received the 9vHPV vaccine or GARDASIL™ in Protocol V503-001. For these subjects, the LTFU study begins once the subject's last visit in the base study is completed.

The LTFU of Protocol V503-001 subjects will be accomplished in 2 ways: registry-based follow-up for effectiveness and safety data, and active follow-up for blood collection at Years 5 and 10 of the LTFU study.

Effectiveness and safety analyses will occur approximately every 2 years following completion of the Protocol V503-001 base study for 10 years. A total of 5 analyses will be summarized in 5 reports (4 Interim Reports and 1 Final Report). The collection and analysis of tissue specimens will be rolled out based upon the expected number of cases. The tissue specimens from Denmark, which has 83% of the subjects, will be collected and analyzed from the beginning of the study. In Norway, which has 14% of subjects, the tissue specimens will be collected and analyzed beginning with the 2nd Interim Report. The tissue specimens from Sweden, with <3% of the subjects, will only be collected and analyzed at the end of the study for the Final Report.

2.4.1.1 Registry Based Follow-Up

For each Interim Report, the Safety Analysis will include all subjects from all 3 countries. The timing of analyses is based on the last subject visit of the Protocol V503-001 base study. In the analyses, a subject's start date in the Protocol V503-021 is their exit date from the Protocol V503-001 base study. A 10 year registry follow-up (to obtain approximately 14 years total follow-up postvaccination) means that 16- to 26-year-old women (Nordic Region enrollment age) will be followed until they are approximately 30 to 40 years old. This period covers the period of peak incidence of CIN 2/3 and AIS, and the onset of the period of highest risk for cervical cancer. Immunogenicity analyses will occur after the Year 5 and Year 10 year study visits are completed.

Merck and the cancer registries have established research contracts for the LTFU study. Currently, the national centralized cervical cancer screening programs in Denmark, Norway, and Sweden recommend for women to have Pap smears collected every 3 years and soon to be added, will be other HPV tests for screening, triage, or diagnosis. When women are overdue for their Pap screening, a reminder letter is routinely sent by each registry and the subsequent reminders are sent until the Pap is completed. This routine procedure is conducted independently of the LTFU study.

Pap tests, other HPV tests for screening, triage, or diagnosis, and histopathology results from biopsies and definitive therapy specimens are collected by subject PIN in national data systems called registries. The registry can obtain biopsy slides and blocks for research analyses. Therefore, the registry data systems can be used to investigate the effectiveness of the 9vHPV vaccine with regard to CIN 1 as biopsy availability allows, CIN 2, CIN 3, AIS, cervical cancer, and vaginal or vulvar cancer, after Protocol V503-001 ends. These data will be entered into the Merck clinical trials database for Protocol V503-021-01.

In addition to the cancer registries, which contain information regarding cervical, vaginal, and vulvar cancers, as well as other HPV tests for cervical screening, triage and diagnosis, these countries have other databases that record health outcomes, such as all deaths, cancers, hospitalizations, and additional safety outcomes as requested by regulatory agencies. Therefore, safety data can also be obtained from other registries to evaluate long-term safety for 10 years following completion of Protocol V503-001 (approximately 14 years following the start of Protocol V503-001). These databases also provide a mechanism to investigate background diseases or immunosuppressive conditions that may be associated with breakthrough HPV disease.

2.4.1.2 Active Follow-Up

In Protocol V503-001, a random sample of approximately 20% of subjects were tested and analyzed for antibody persistence during the study. Those subjects from Denmark who continue in Protocol V503-021 and were included in this analysis of antibody persistence in Protocol V503-001 will continue to be tested and analyzed for antibody persistence in Protocol V503-021. These subjects in Denmark will be contacted to visit a blood collection site for 2 blood collection visits. The study visits will occur

approximately 5 years and 10 years into the LTFU study. Serum will be analyzed for anti-HPV responses as described in Section 2.5.1.

2.5 LIST OF IMMUNOGENICITY AND EFFICACY MEASUREMENTS

2.5.1 Immunogenicity Measurements

Serum obtained at Year 5 and Year 10 will be analyzed for anti-HPV type 6, 11, 16, 18, 31, 33, 45, 52, and 58 levels. Serum may be analyzed using a variety of HPV immunoassays and for other HPV types. Serum geometric mean titers (GMTs), and seropositivity rates to HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 will be summarized after the LTFU 5-year and 10-year study visits. The results will be analyzed as exploratory, together with data from the primary follow-up period of Protocol V503-001 data, to assess long-term antibody responses.

2.5.2 Efficacy Measurements

Long-term effectiveness will be assessed by determining the incidence of HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 related pre-malignant and malignant genital tract disease in vaccine recipients from Protocol V503-001. The NPP diagnosis will provide the official diagnosis for endpoints. (See Section for 3.2 for other roles and responsibilities.)

Those vaccinated with the 9vHPVvaccine comprise a sentinel cohort that will provide approximately 5 years lead-time over the general population for identifying potential vaccine breakthroughs due to waning effectiveness. Such breakthroughs will be identified by obtaining tissue blocks from biopsy and definitive therapy specimens and testing these tissue specimens by thinsection PCR. Threshold levels of breakthrough disease have been established to define when a need for a booster dose of vaccine may exist (see Section 3.5.3.1).

2.6 LIST OF SAFETY MEASUREMENTS

Long-term safety will be assessed by health outcomes, including deaths, cancers, and hospitalizations identified in various healthcare registries. Because data acquisition is passive, relatedness will not be determined in the context of the LTFU study. This is a non-interventional protocol; therefore serious and non-serious adverse experiences will not be solicited. Only procedure-related serious adverse experiences will be collected. In this study, the only protocol-specified procedure is serum collection at Year 5 and Year 10.

2.7 STATISTICAL ANALYSIS PLAN SUMMARY

In the absence of a control group in the LTFU study, it is not possible to formally test the effectiveness hypotheses. Instead, effectiveness will be measured in terms of incidence rates of disease, with analyses performed periodically until the end of the follow-up in 2024. All recipients of the 9vHPVvaccine in Protocol V503-001 from the participating Nordic countries who consented to the LTFU study will contribute to the effectiveness analyses (Cohort 1). At each analysis time point, the cumulative and current (for the

current interval) incidence rate for each effectiveness endpoint will be computed along with the associated 95% confidence interval (CI).

As an aid to interpretation, the expected incidence of disease in an unvaccinated cohort was carefully estimated. Historical data from the national registries was combined with survey data to estimate the total incidence of CIN 2/3 or AIS (regardless of HPV type-relatedness), in a population with a level of sexual activity approximating that of the Protocol V503-001 cohort. The estimated incidence of CIN 2/3 or AIS was 5.48/1000 person-years. Then, the proportion of disease related to HPV 16/18/31/33/45/52/58 was estimated based on published data on HPV type prevalence in CIN 2/3 or AIS lesions. This estimate was 80%. Therefore, the estimated incidence rate of CIN 2/3 or AIS attributed to HPV 16/18/31/33/45/52/58 is 4.38/1000 person-years. This estimate will be used to evaluate vaccine effectiveness.

The primary analysis approach will be per-protocol. To be eligible for this population, subjects must (i) have received 3 doses of the 9vHPVvaccine within 1 year, and (ii) have no protocol violations. Subjects will be considered cases related to a given HPV type provided the subject was negative to the respective HPV type by serology and PCR prior to vaccination, and PCR-negative through Month 7. Due to the Month 7 PCR criterion, only Cohort 1 will be considered in the per-protocol primary analysis. For purposes of endpoint definition, only the NPP diagnosis will be considered.

A control chart will be used to provide indications that vaccine effectiveness is waning. Upper monitoring bounds, or control limits, will be established to indicate whether the current incidence of breakthrough disease is exceeding 10% of the estimated incidence in an unvaccinated cohort of similar age and risk level. Assuming the incidence rate for HPV 16/18/31/33/45/52/58-related CIN 2/3 or AIS in unvaccinated subjects is 4.38/1000 person-years, the target incidence rate in vaccinated subjects is 0.438/1000 person years. Further, assume that approximately 1900 subjects in Cohort 1 are expected to be eligible for LTFU primary effectiveness analysis in the Per-Protocol Efficacy (PPE) population based on attrition during, Protocol V503-001 base study follow-up, ineligibility for the analysis population, or unwillingness to consent to LTFU. Then if (1) the number of breakthrough cases exceeds the 2.75-sigma control limit based on the target incidence rate once in any analysis, or (2) the number of breakthrough cases exceeds the 1.83-sigma control limit based on the target incidence rate on 2 occasions over 3 consecutive intervals in any analysis for the primary endpoint, then discussions will be held with regulatory agencies on potential actions to be taken with regard to then waning effectiveness.

The sample size for the effectiveness and immunogenicity components of the LTFU study is fixed by the number of Protocol V503-001 study participants who are eligible and willing to participate in the LTFU study. There are 4453 subjects total in Cohorts 1 and 2. Only those subjects in Cohort 1 are considered in the performance assessment of the control chart, since these subjects will contribute to the primary effectiveness analysis in the PPE population. As stated above, approximately 1900 subjects in Cohort 1 are expected to be eligible for the LTFU primary effectiveness analysis in the PPE

population. Using the signaling rule stated above leads to an approximate alpha-level of 0.078 (approximate 95% CI: 0.062, 0.094), if vaccine effectiveness is at least 90% over the follow-up period. The same signaling rule provides approximately 95.2% (approximate 95% CI: 93.9%, 96.5%) power to detect a decrease in vaccine effectiveness for the primary endpoint in the PPE population, if effectiveness decreases from 90% to 50% linearly over the LTFU period.

Cohort 2 will consist of subjects who have either received GARDASIL™ only and/or subjects who received GARDASIL™ followed by the 9vHPVvaccine, depending on whether the subjects in the V503-001 base study are offered the 9vHPVvaccine as part of a study extension. These subgroups of Cohort 2 may be analyzed separately for exploratory analyses if data are available in each subgroup. The vaccine doses received will need to be considered when interpreting the efficacy analyses for this cohort. Subjects in Cohort 2 vaccinated with the 9vHPVvaccine will have approximately 5 more years of potential exposure than subjects in Cohort 1 to HPV types 31, 33, 45, 52, and 58 prior to vaccination, and their HPV exposure prior to vaccination may not be fully understood. Therefore, results from Cohort 2 effectiveness analyses may be difficult to interpret.

3. PROTOCOL DETAILS

3.1 RATIONALE

3.1.1 Rationale for This Study

In Denmark, Norway, and Sweden, cervical cancer screening and registry surveillance systems routinely collect Pap, cervical biopsy, and definitive therapy results, as well as, vaginal and vulvar cancer diagnoses. These countries anticipate updating their recommendations in the coming years to include additional HPV tests for screening, triage, and diagnosis. Data collection of all test results in these registries is mandated by law; therefore, the completeness of data for each field collected approaches 100%.

Each registry holds the linkage between the AN and PIN, and can be used to search for disease outcomes in study participants. Tissue blocks are also available from the registry surveillance systems and have been used for HPV PCR testing in Protocol V501-015 and will be similarly available in the Protocol V503-021 LTFU study. In this V503-021 LTFU study, the HPV types that will be tested by PCR are the 9 vaccine types, plus 5 other oncogenic types, HPV 35, 39, 51, 56, and 59.

In each country, all Pap tests, as well as other HPV tests for screening, triage and diagnosis, whether collected from the cancer screening program or from opportunistic Pap or other HPV tests done by practitioners, are captured in the national registries. Both normal and abnormal (CIN 1, CIN 2, CIN 3, AIS, or cervical cancer) biopsy results and vulvar and vaginal cancers are collected in these national registries. However, there are minor differences among countries in the starting age for cancer screening (23 years of age in Sweden and Denmark; 25 years of age in Norway). These differences are not expected to affect ascertainment in the LTFU study because the subjects are already heavily screened within the context of the Protocol V503-001 base study and are currently within screening age range.

A number of relevant epidemiologic studies have been performed in the Nordic region by Merck Research Laboratories (MRL) and local investigators. One MRL study in particular, the Concomitant Cohort Study (CCS), is highly germane to the LTFU study, because it provided background rates of CIN in a population that controls for important covariates, to guide the estimates of expected incidence of disease in Protocol V503 021 going forward [1].

The LTFU study is designed to accomplish the following major objectives:

- To evaluate the long-term effectiveness of 9vHPVvaccine in Protocol V503-021 subjects from the relevant countries by searching for possible breakthrough disease from vaccine-related HPV types
- To study possible HPV type replacement effects in non-vaccine HPV types
- To characterize the long-term antibody response
- To assess long-term safety

Long-Term Effectiveness

Long-term effectiveness will be assessed by determining the incidence of HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 related pre-malignant and malignant genital tract disease in vaccine recipients from the Protocol V503-001 base study. The 9vHPVvaccine recipients are a sentinel cohort that provides approximately 4 years lead time for identifying potential vaccine breakthroughs. Such breakthroughs will be identified by obtaining the tissue blocks from biopsy and definitive therapy specimens and testing tissue sections by thinsection PCR. Threshold levels of incidence of breakthrough cases have been established to define a point where the need for a booster dose of vaccine may exist, and where vaccine effectiveness may have waned by a moderate amount. Crossing the threshold will not necessarily indicate that the effectiveness is at an unacceptable level, however.

HPV Type Replacement with Non-vaccine Types

As time after vaccination passes and an increasing proportion of the population is vaccinated, it is expected that the overall incidence of HPV vaccine types would decrease. HPV type replacement is not expected to occur, but will be explored in high-grade lesions from the PPE analysis population [2].

Protocol V501-005 lends itself to a study of possible type replacement because it was the prototype study that used monovalent HPV type 16 VLP vaccine [3]. Therefore, incidence of HPV type 6, 11 and 18-related detection, infection, CIN, and external genital lesions incidence could be compared in the vaccine and placebo groups to identify possible HPV type replacement over the 4 years of the study. Within each of the HPV types examined, analysis was conducted among subjects who received all 3 vaccinations, did not deviate from the protocol in ways that could interfere with the detection of the endpoint, and were PCR- negative Day 1 through Month 7 for the relevant HPV type(s). Analysis showed that the incidence rates of persistent HPV 6, 11, and/or 18 detection or infection in the 2 vaccination groups were comparable, as were incidence rates of HPV 6, 11, and/or 18 related external genital or cervical disease.

Immunogenicity

Long term immunogenicity will be assessed by continuing effectiveness and by obtaining serum for competitive Luminex Immunoassay (cLIA) testing at Year 5 and Year 10 from a subset of subjects in Denmark. Serum will also be tested using total immunoglobulin G (IgG) immunoassay or other serological assays.

Safety

On a periodic basis, the NRSCs will search their appropriate registries for hospital discharge information pertaining to all deaths, cancers, hospitalizations and additional safety outcomes as requested by regulatory agencies for each subject who has consented to enrollment in the LTFU study. Similar to the GARDASIL™ long-term follow-up study, specific disorders that will be evaluated may include, but are not limited to,

incident cases of: systemic lupus erythematosus, rheumatoid arthritis, Guillain-Barre syndrome, acute disseminated encephalomyelitis, and multiple sclerosis in hospital discharge records. The results of these searches will be summarized as new medical history and provided to the SPONSOR. Descriptive comparisons to rates of similar conditions in the unvaccinated general population will be done as described in Section 3.5.5.3.

3.2 STUDY PROCEDURES

3.2.1 Summary of Scheduled Study Visit Procedures

The country specific Study Flow Charts summarize procedures for scheduled study visits. Activities conducted during scheduled study visits include obtaining informed consent (which should be done before completing the base study) and collecting blood samples from a subset of subjects for immunogenicity measurements. This section provides clarifications to the scheduled study visit procedures.

3.2.1.1 Informed Consent

All subjects participating in the Protocol V503-001 base study, and who are living in Denmark, Norway, and Sweden will be eligible to participate in the LTFU study. Most of the eligible subjects have consented to the follow up portions of the Protocol V503-021 LTFU study as part of the Protocol V503-001 base study and by any additional country-specific consent requirements.

The subject's consent for the LTFU study will permit the NRSC and/or study site to contact participating subjects for collection of serum and will allow the SPONSOR to review the subject's registry database information, and to obtain the following records and specimens:

1) Pap test (cytology) results and other HPV tests for screening, triage, and diagnosis

2) Gynecologically-related biopsy or definitive therapy (pathology) results and reports

3) Gynecologically-related specimen H&E slides

4) Gynecologically-related specimen blocks

5) Serology at 5 years and 10 years following the end of Protocol V503-001

6) Nationally banked serum specimens, if available

7) Allow for research in registry database for safety, review of hospital records

Subjects may change their consent for the LTFU activities during the course of the study, and subjects may withdraw at any time.

3.2.1.2 Calculation of Scheduled Visit Windows

There are no protocol-defined windows for the serum collection visit. Samples from a subset of subjects from Denmark will be collected during two study-wide blood collection campaigns: one approximately 5 years and one approximately 10 years following the start of the LTFU study.

3.2.1.3 Serum for Antibody Measurements

During the course of the LTFU study, a subset of subjects from Denmark will be requested to complete 2 study visits at approximately 5 years and 10 years following completion of the Protocol V503-001 base study. The 2 study visits will only require serum collection. The NRSC will determine the location(s) available for subjects to visit for serum collection and will notify the subjects of available locations. It is possible that these locations may differ from that of the subject's primary care physician. The NRSC will be responsible for notifying the subjects approximately 6 months prior to their scheduled visit.

Materials and labels needed for this collection will be provided to the NRSC by the SPONSOR and distributed to the serum collection locations. For collection of serum, the serum collection locations must follow the procedures described in this protocol and the Administrative Binder, and the serum collection locations must use the materials provided by the SPONSOR (see Section 3.2.3.1).

All serology specimens will be analyzed for anti-HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 using a variety of assays.

A 10-mL blood specimen will be collected, and serum separated avoiding any hemolysis. All sera should be stored in a freezer at -20°C or below until shipped frozen on dry ice.

3.2.2 Summary of Unscheduled Study Visit Procedures

3.2.2.1 Ascertainment of Pap Tests, Other HPV Screening Tests, Biopsy, and Definitive Therapy Results

Periodically, following completion of the Protocol V503-001 base study, the NRSCs will search their respective national cancer registries for results of Pap tests, other HPV tests for screening, triage, and diagnosis, cervical biopsies, definitive therapy procedures, and vaginal or vulvar cancers. The first search of the registries will cover the time period from the first patient out of the Protocol V503-001 base study until approximately 24 months after the last patient out (LPO). All of the Nordic Cohort I Subjects completed the V503-001 base study by the end of 4Q2013. Because the registries capture the identity of the laboratory from which the report was originally generated, it will be possible to filter out any results originating from the Protocol V503-001 base study central laboratory for active subjects. The subsequent registry searches will be conducted following the end date of the previous search. Required fields in the search include subject identifiers, date of examination, diagnosis, and laboratory where the pathology reading was made.

Primary diagnoses for biopsies, Paps and other HPV tests for screening, triage, and diagnosis from the registry searches will be documented in the study database. The procedures for sending tissue for biopsies to the SPONSOR are similar to events that have occurred outside the context of the study for the HPV vaccine program. The details of these procedures can be found in the Pathology Panel Standard Operating Procedure (SOP).

3.2.2.2 Processing of Biopsy and Definitive Therapy Pathology Reports, H&E Slides, Specimen Blocks and Thinsection PCR

All biopsies and definitive therapy samples will have been initially processed per local standards. The SPONSOR will learn of all cervical biopsies, endocervical curettage (ECC) samples, definitive therapy samples, and vaginal or vulvar biopsies resulting in vaginal or vulvar cancer, respectively, only after the local NRSC has searched their respective registry databases. The NRSC will be responsible for obtaining the tissue blocks, H&E slides, and pathology reports from the local laboratories. The NRSC will rout the blocks to the pathology lab and the H&E Slides and pathology reports to the Nordic Coordinating Center (NCC), a central group who will be responsible for the administrative processes related to the Pathology Panel. The original set of H&E slides, along with a new set of H&E slides, will be read by the NPP, a group of 4 expert pathologists from the region. The detailed instruction for routing these samples will be written prior to reading the first pathology slide for the study. The Pathology Panel SOP will be approved by the NPP and the SPONSOR. As was done in the GARDASIL LTFU study, a validation plan will be implemented by using standard pathology slides to assess agreement between pathologists, and possible changes in reading criteria over time..

3.2.2.3 Ascertainment of Specific Safety Data

On a periodic basis, the NRSCs will search their appropriate registries for information pertaining to all deaths, cancers, hospitalizations, and additional safety outcomes as previously requested by regulatory agencies for GARDASIL™ for each subject who has consented to the LTFU study. Specific disorders that will be evaluated may include, but are not limited to, incident cases of: systemic lupus erythematosus, rheumatoid arthritis, Guillain-Barre syndrome, acute disseminated encephalomyelitis, and multiple sclerosis in hospital discharge records. The results of these searches will be summarized and provided to the SPONSOR. Descriptive comparisons to rates of similar conditions in the unvaccinated general population will be done as described in Section 3.5.5.3.

3.2.3 Procedures for Collection and Handling of Study Specimens

For scheduled study visits, consult the country specific Study Flow Charts for the specific samples needed. The following are the step-by-step procedures for collection of study specimens, a description of the supplies needed, and the guidelines for handling specimens.

3.2.3.1 Serum for Anti-HPV Measurements at Scheduled Visits

For each visit that requires a serum specimen for anti-HPV measurements, a 10-mL (non-heparinized, non-serum separator, red-top tube provided by the SPONSOR) blood specimen will be collected and should be separated to avoid hemolysis. A minimum of 3.0 mL of serum should be aliquoted to a vial provided by the SPONSOR and labeled with the "Serum" label provided by the SPONSOR. An additional 1.5 mL of serum, a "Retention Serum", should be aliquoted to a vial provided by the SPONSOR and labeled with the "Retention Serum" label provided by the SPONSOR. Within 30 minutes of collection, place the Serum and Retention Serum in a freezer at -20°C (or lower) until the samples are shipped on dry ice as instructed by the SPONSOR (See Administrative Binder). Serum and Retention Serum should be shipped separately.

If the samples thaw, contact the SPONSOR. Thawed serum samples require written documentation, including details such as allocation number, date of collection, and length of time sample was exposed to temperature excursion (see the Administrative Binder for a summary of deviations that require documentation in this study). Further information regarding handling, labeling, and shipping of samples are given in the Administrative Binder.

All available serum should be used for conducting assays specified in the clinical protocol. Serum and Retention Serum may also be used for further HPV immunologic testing in addition to tests specified in the protocol. Serum testing is to be completed before the end of the study (final report of study results).

3.2.4 Allocation

A single subject cannot be assigned more than 1 allocation number. The Allocation Number assigned to the subjects in the base study will be retained by the subjects in the long-term follow-up study.

3.2.5 Discontinuation/Withdrawal from Study

Subjects/patients may withdraw at any time by contacting the original study site. Any adverse experiences which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 3.4 SAFETY MEASUREMENTS - DETAILS.

3.2.6 Subject Relocation

If a subject relocates to one of the other participating countries in this study, reasonable efforts will be made to obtain specimens and data from that country.

3.3 EFFICACY/IMMUNOGENICITY MEASUREMENTS

3.3.1 Immunogenicity Measurements

The 9-valent HPV cLIA and 9-valent HPV total IgG Luminex Immunoassay are the primary assays used for the primary objective of the study. Additional testing may be conducted on a subset of subjects using another HPV immunological assay

(Pseudovirion-based Neutralization Assay, or PBNA) for supportive exploratory analyses.

3.3.1.1 Competitive Luminex Immunoassay (cLIA) - Anti-HPV Levels in Serum

The purpose of the 9-valent HPV cLIA(HPV9 cLIA) is to measure antibodies to HPV VLPs, types 6, 11, 16, 18, 31, 33, 45, 52 and 58 before and after vaccination with the HPV 9-valent vaccine. This assay is used to evaluate the serological response following vaccination and to measure HPV infection induced antibodies for seroepidemiology studies.

Yeast-derived VLPs are coupled to a set of nine distinct fluorescent Luminex microspheres. Antibody titers are determined in a multiplexed, competitive format in which known, type-specific phycoerythrin (PE)-labeled, neutralizing monoclonal antibodies (mAbs) compete with the subject's serum antibodies for binding to type-specific, conformationally sensitive, neutralizing epitopes on the VLPs. The fluorescent signals from the bound HPV-specific detection mAbs are inversely proportional to the subject's neutralizing antibody titers. Results for the assay are reported as concentration of antibody in arbitrary milli-Merck Units per milliliter (mMU/mL).

The HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 cLIA is performed in a 96-well microtiter plate. A 12-point standard reference serum pool from adult females vaccinated with a 9-valent vaccine, 4 controls, and 16 samples are added to the plate in duplicate. Samples are tested at a 1:4 and a 1:40 dilution. To each well is added the detection antibodies followed by the VLP-microspheres for types 6, 11, 16, 18, 31, 33, 45, 52 and 58. The plates are sealed with foil covers and incubated for 15 to 25 hours. Following incubation, the plates are washed 3 times and the samples are analyzed on a BioPlex (Luminex) instrument.

The high, medium, low and negative controls used for this assay were collected from humans that were either HPV-seronegative, had low antibody titers from natural infection, or had medium-to-high antibody titers to the nine HPV types following vaccination.

3.3.1.2 Total IgG Luminex Immunoassay

The purpose of the 9-valent HPV total IgG Luminex immunoassay (HPV9 IgG) is to measure antibody concentrations to HPV VLPs types 6, 11, 16, 18, 31, 33, 45, 52 and 58 before and after vaccination with the 9-valent HPV vaccine. This assay is used to evaluate the serological response following vaccination and to measure HPV infection-induced antibodies for seroepidemiology studies.

Yeast-derived VLPs are coupled to a set of nine distinct fluorescent Luminex microspheres. Antibody concentrations are determined in a multiplexed, direct-binding format by measuring the amount of VLP-specific IgG bound to VLP-microspheres. Following incubation with human serum, fluorescent signal from an anti-human IgG detection antibody that binds directly to serum IgG and equally to each IgG subclass (1 to

4), is directly measured on the Luminex or BioPlex instrument. The fluorescent signal from the IgGbound fluorescent detection antibody is proportional to the individual's anti-VLP IgG antibody levels. Results for the assay are reported as concentration of antibody in arbitrary mMU/mL.

The HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 IgG assay is performed in a 96-well microtiter filter plate. A 12-point standard reference serum pool from adult females vaccinated with a 9-valent vaccine, 4 controls, and 16 samples are added to the plate in duplicate. Samples are tested at a 1:100 and a 1:10,000 dilution. To each well is added the VLP-microspheres for types 6, 11, 16, 18, 31, 33, 45, 52 and 58. The plates are sealed with foil covers and incubated for 15 to 60 minutes. The contents of the filter plate are washed and incubated with the mouse, anti-human IgG_{1-4} mAB conjugated to PE. The plates are covered with foil and incubated for an additional 30 to 60 minutes. Following the second incubation period, the plates are washed 3 times and the samples are analyzed on a BioPlex (Luminex) instrument.

The high, medium, low and negative controls used for this assay were collected from humans that were either HPV-seronegative, had low antibody concentrations from natural infection, or had medium-to-high antibody concentrations to the nine HPV types following vaccination.

3.3.1.3 Pseudovirion-based Neutralization Assay

The purpose of the HPV 16 and HPV 18 PBNA are to detect the presence of antibodies capable of inhibiting cellular uptake of HPV pseudovirions for HPV types 16 and 18 in serum after vaccination with the 9-valent HPV vaccine. This assay is developed and executed by Deutsche Krebsforschungszentrum (DKFZ) laboratories, Heidelberg, Germany, on behalf of MRL to evaluate neutralizing antibody response to the HPV 16 and 18 components of the vaccines.

HPV 16 and 18 pseudovirions are produced at DKFZ laboratories by co-transfecting the 293TT human embryonic kidney cell line with an expression plasmid encoding the HPV L1 and L2 capsid genes and another encoding luciferase from the marine copepod *Gaussia princeps*. Pseudovirions of L1/L2 self-assemble and package the luciferase reporter plasmid within. Pseudovirions are incubated with HeLaT K4 cells and, when pseudovirions are able to enter cells, *Gaussia* luciferase is expressed and secreted to the cell culture supernatant. If neutralizing antibodies are present in the test sera, infection of cells by pseudovirions and subsequent expression of luciferase reporter is inhibited. The addition of luciferase substrate, coelenterazine, to the reaction results in luminescence when luciferase is present in the cell culture supernatant. This luminescence is measured in a plate reader.

The PBNA assay is performed in a 384-well format in clear, flat-bottom culture plates. Sera and controls are initially diluted 1:2.5 in neutralization cell culture medium and serially diluted 4-fold in a master plate from which nine identical assay plates are aliquoted to allow for triplicate measurements of neutralization for each: HPV16, HPV18, and BPV (bovine papillomavirus) pseudovirions. BPV PBNA assays are run as

a control to verify that the test serum is not toxic to the cells, which can mimic neutralization. Pre-prepared serum assay plates are thawed and diluted pseudovirions are added, 15µL/well, such that the final dilutions of pseudovirions in each assay after addition of cells are: 1:20,000 for HPV16, 1:40,000 for HPV18 and 1:80,000 for BPV. Plates are incubated at room temperature for 1 hour. Human HeLaT K4 cells, 20 µL/well, are seeded onto the plates in neutralization cell culture medium at a density of 1500 cells/well and incubated at 37°C and 5% CO_2 for 2 days. The triplicate HPV16, HPV18 and BPV PBNA assay plates are equilibrated to room temperature before addition of 1:100 diluted coelenterazine substrate buffer to each well of each plate, 20 µl/well, using FlexDrop automation, which synchronizes substrate addition to allow equal incubation times of all 9 plates in a batch. Luminescence is read by an Envision 2101 plate reader and data are stored as a text file. Serum neutralization titers are calculated by linear interpolation and defined as the reciprocal of the serum dilution that caused 50% reduction in luciferase reporter activity (EC_{50}) when compared to control wells (pseudovirions in the absence of serum and pseudovirions in the presence of a standard serum derived from HPV vaccine recipient).

This assay is currently designated to provide a secondary measurement, complementary to the 9-valent HPV cLIA and 9-valent HPV IgG assay.

3.3.2 PCR Assays - Detection of HPV in Tissue Specimens

Thinsection microtomy biopsy specimens will be tested for detection of HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. In addition to this testing, Thinsection microtomy biopsy specimens may be tested for other HPV types.

HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 will be analyzed by type-specific multiplex (L1, E6, E7 gene detection) PCR assay (described in Section 3.3.2.1). HPV types other than 6, 11, 16, 18, 31, 33, 45, 52, and 58 will be analyzed by the duplex (E6, E7 gene detection) PCR assay (using the preparation method described in Section 3.3.2.1).

3.3.2.1 Multiplex PCR Assays

The following procedures will be done for the detection of HPV Types 6, 11, 16, 18, 31, 33, 45, 52, and 58 in frozen swabs and Thinsection microtomy biopsy samples. Specimens are received and then prepared for multiplex PCR using a DNA purification method (Qiagen Technology Kit). Multiplex PCR (based on real-time fluorescent PCR) allows the simultaneous detection of 3 gene products (L1, E6, and E7) for a given HPV type in 1 reaction. The HPV type-specific primer pairs based on the published HPV L1, E6, and E7 sequences, are used to specifically amplify a portion of each gene simultaneously. The specific amplicons are detected in real-time by fluorescently-labeled oligonucleotide probes. The gene-specific oligonucleotide probes are each labeled with a different fluorescent label, and the fluorescent emission is captured during PCR cycling.

After analysis of the raw fluorescent data by the real-time PCR instrument software, a threshold cycle (Ct), which represents the PCR cycle at which an increase in reporter fluorescence above a baseline signal can first be detected, is determined. Each gene-

specific assay (i.e., gene-specific dye layer) is considered positive if the Ct is <45 cycles. A gene-specific assay is considered negative if the Ct = "No Ct". A sample is called positive when 2 or 3 genes are positive or when the same single gene scores positive on consecutive tests.

3.3.2.2 Preparation and Disposition of Thinsections of Biopsy Tissue

The following procedures will be performed at the SPONSOR-designated Pathology Laboratory. The procedures will be performed by an experienced, qualified histotechnologist according to the Pathology Laboratory's SOP. The histotechnologist will assure that the microtome and work areas are clean and free of contaminants. All Thinsection microtomy for PCR will be performed at a time when all other routine work has been completed, so that potential contamination can be minimized. Prior to sectioning each block, a new blade will be installed in the microtome. The block will only be positioned so that it is at the left margin of the blade surface. Technicians sectioning study blocks will utilize “biologically clean” gloves while handling the blocks (new gloves for each block). First, the histotechnologist will face the block by removing two 4-micron sections from the face of the block. These sections will be discarded. Using sterile plastic forceps, the next two 4-micron paraffin sections are collected and floated in a water bath for the preparation of 1 H&E slide (Slide 1, with 2 sections).

Nine additional, consecutive sections will then be cut to be used for Thinsection PCR. There will be 9 individual tubes (Tube 1, 2, 3, 4, 5, 6, 7, 8, 9), and one 4-micron section will be placed in each tube using a sterile disposable plastic forceps. The pair of sterile plastic forceps used is then discarded after placing the cut section in each tube. Each tube is then placed inside a plastic sleeve and sealed.

Two additional, consecutive 4-micron sections will then be cut and the 2 sections floated in the water bath for preparation of the second H&E slide, both sections to be placed on one slide (Slide 2 with 2 sections each). All H&E slides (Slides 1 and 2) will have a histopathologic review by the laboratory's pathologist.

Slides and tubes should be labeled with the subject's allocation number. The specimen tubes are collated with the appropriate specimen requisition and prepared for shipping to the NCC and/or SPONSOR-designated Laboratory.

The microtome is cleaned in preparation for the next block and the process above is repeated. The microtome blade is replaced with a new blade and adjusted for each new biopsy block and the same procedure is to be followed. A new pair of clean gloves and a new pair of clean, disposable forceps will be used for each block being sectioned. The “used” blade may be retained for cutting non-PCR blocks. The total number of sections to be cut from each block is 13. A total of 2 slides and 9 tubes:

1. Slide 1 (H&E), with 2 sections each, stained.
2. Tubes 1, 2, 3, 4, 5, 6, 7, 8, 9 (HPV PCR Analysis), 1 section per tube.
3. Slide 2 (H&E), with 2 sections each, stained.

3.3.3 Conduct of the Clinical Trial

The clinical effectiveness diagnoses will be adjudicated by the Nordic Pathology Panel (NPP) responsible for the definitive pathologic diagnoses in all clinical conditions which may be considered as possible endpoints in subjects in this trial. The operation of the NPP is described below.

3.3.3.1 Responsibility of the Nordic Pathology Panel

The clinical effectiveness endpoints will be adjudicated by the Nordic Pathology Panel (NPP), responsible for the definitive pathologic diagnoses in all clinical conditions which may be considered as possible endpoints in subjects in this trial.

The NPP will be responsible for providing the definitive pathologic diagnoses of cervical biopsies, ECC specimens, vaginal biopsies, vulvar biopsies, and definitive therapy specimens for the purpose of determining the presence of endpoints in the study (not for medical management). Slides from cervical, vulvar, and vaginal biopsies and definitive therapy will be evaluated by the NPP. The NPP will prepare reports on each tissue specimen.

The activities and responsibilities of the NPP for this study will be detailed in SOPs as was done for the GARDASIL™ LTFU study (Protocol V501-015-21). Included in an SOP will be the use of a "Safety Net Letter" to inform the Principal Investigator that a Pathology Panel histological classification of a biopsy specimen is more severe than the Central Laboratory pathologist’s diagnosis. No mandatory action will be required for any lesion, if the diagnosis of the local (i.e., non-study) laboratory and the diagnosis from the NPP are different grades.

3.3.4 Adjudication Procedures

Specific details regarding endpoint definitions can be found in Section 3.5.

3.4 SAFETY MEASUREMENTS

The sections below summarize the definition of, and reporting requirements for an adverse event (AE), serious adverse event (SAE), non-serious adverse event (NSAE) and attributed SAE, that are generally applicable to non-interventional studies. This is a non-interventional protocol, so AE's or NSAE's will not be solicited. Only procedure- related SAE's will be collected. In addition, from the registries, other safety outcomes, such as deaths, cancers, and hospitalizations will be collected as described in Section 2.4.1.1.

3.4.1 Serious Adverse Event Reporting

This is a non-interventional study. No individual administration of any therapeutic or prophylactic agent is assigned in this protocol.

If through the conduct of this study, an investigator becomes aware of any SAE, regardless of attribution, which occurs in any study subject within 5 days following a protocol-specified blood draw, the Investigator must report it to the sponsor.

Additionally, any SAE brought to the attention of an investigator at any time after the above specified time period must be reported to the Sponsor if the event is attributed to the protocol-specified blood draw procedure.

Also required to be reported is any SAE that is attributed to the 9vHPV vaccine or any other investigational or marketed product manufactured by Merck.

Upon becoming aware of the SAE, the INVESTIGATOR will enter the SAE information directly into the Electronic Data Capture System within 24 hours. Refer to the Electronic Case Report Form (eCRF) Entry Guidelines for what information must be reported.

All subjects with SAEs related to protocol-specified blood draw procedure must be followed up for outcome.

3.4.1.1 Non-Serious Adverse Event Reporting

Although NSAEs are not actively solicited in this study, if any attributed NSAEs are reported by the investigator, they must be submitted to Global Safety within 10 calendar days using the same method as described above for SAEs. The attributed NSAEs will be tabulated and included in the interim and/or final study report.

3.4.2 Definition of Adverse Event, Attributed Adverse Event and Serious Adverse Event

3.4.2.1 Adverse Event

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product or who undergoes a protocol-specified procedure and which does not necessarily have to have a causal relationship with this treatment or procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Sponsor's product, is also an AE.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered AEs. Examples of this may include, but are not limited to, teething, typical crying in infants and children, and onset of menses or menopause occurring at a physiologically appropriate time.

Sponsor's product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator product) or marketed, manufactured by, licensed by, provided by or distributed by the Sponsor for human use.

AEs may occur during the course of the use of the Sponsor's product in studies or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

3.4.2.2 Attributed Adverse Event

An attributed AE is an AE that is felt to be causally related to a Sponsor's product. During studies with direct patient contact (visits), the assessment of causality will be determined by an investigator who is a qualified physician according to his/her best clinical judgment. Use the following criteria as guidance (not all criteria must be present to be indicative of attribution to a Sponsor's product: There is evidence of exposure to the Sponsor's product; the temporal sequence of the AE onset relative to the administration of the Sponsor's product is reasonable; and the AE is more likely explained by the Sponsor's product than by another cause. In studies without direct patient contact, the assessment of causality would be determined by a notation of attribution in medical records. Attribution can be assigned by the investigator or the Sponsor. Examples include a drug-induced rash that an investigator attributes to a specific product, or a clinical notation that a product was discontinued because it caused insomnia.

3.4.2.3 Serious Adverse Event

An SAE is an AE which is fatal or life threatening, results in persistent or significant disability/incapacity, requires inpatient hospitalization, prolongation of existing inpatient hospitalization, or is a congenital anomaly/birth defect, cancer, the result of an overdose or is another important medical event. Other important medical events that may not result in death, may not be life-threatening, or may not require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the other outcomes listed previously. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home and blood dyscrasias or convulsions that do not result in inpatient hospitalization.

3.4.2.4 SAE/Attributable NSAE in Study Reports

The end-of-study report, and any interim analysis, will include aggregate listings of all SAEs and any spontaneously reported NSAEs attributable to the 9vHPVvaccine and will be provided to regulatory agencies as required. All interim and final study reports will be summarized in Periodic Safety Update Reports (PSUR's) and/or Development Safety Update Reports (DSUR's) until completion of the study as required.

SAEs and spontaneously reported NSAEs attributable to OTHER investigational or marketed products manufactured by the Sponsor will be collected and reported to regulatory agencies as individual cases as required but will not be included in the study’s final or interim reports.

3.4.3 Reporting of Overdose to Sponsor

No overdose should occur since no subject will be administered vaccinations of 9vHPVvaccine or GARDASIL™ vaccine during this study.

3.4.4 SPONSOR Responsibility for Reporting Adverse Experiences

All adverse experiences will be reported to regulatory agencies, IRB/IECs, and investigators in accordance with all applicable global laws and regulations.

3.5 STATISTICAL ANALYSIS PLAN

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, changes are made to the primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to the exploratory or other non-confirmatory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be listed in the study report. Post hoc exploratory analyses will also be clearly identified in the study report. No separate Statistical Analysis Plan (SAP) will be issued for this study.

3.5.1 Responsibility for and Timing of Analyses

The statistical analysis of the data obtained from this study will be the responsibility of the Clinical Biostatistics department of the SPONSOR. All analyses will be done in collaboration with the NRSCs.

This extension study will not be blinded since all participants will have previously participated in the Protocol V503-001 base study and will be unblinded.

In the Nordic region, effectiveness analyses will occur approximately 2 years following the completion of Protocol V503-001, and approximately every 2 years thereafter, for a total of 5 analyses. Indications of breakthrough disease may increase the frequency of analyses of effectiveness. Immunogenicity analyses will occur after the 5- and 10-year study visits following completion of Protocol V503-001.

3.5.2 Hypotheses

The study hypotheses are listed in Section 2.1 of the protocol. Due to the absence of a control group, the hypothesis is not formally controlled by statistical power and significance levels.

3.5.3 Variables and Time Points of Interest

3.5.3.1 Effectiveness

A primary effectiveness case is defined as a subject who is found to have an incident case of HPV 16/18/31/33/45/52/58-related CIN grades 2 or 3, AIS, or cervical cancer. This is defined to have occurred when on a single cervical biopsy, ECC, Loop Electrosurgical Excision Procedure (LEEP) or Conization (cold knife/laser) specimen, there is an NPP

consensus diagnosis of CIN 2, CIN 3, AIS, or cervical cancer and at least 1 of HPV types 16, 18, 31, 33, 45, 52, or 58 is detected by Thinsection PCR in an adjacent section from the same tissue block.

The secondary effectiveness endpoints are as follows:

1. *CIN 2, CIN 3, AIS, or cervical cancer related to HPV 35, 39, 51, 56, or 59*. This endpoint is defined to have occurred if on a single biopsy or excised tissue, there is the NPP consensus diagnosis of CIN 2, CIN 3, AIS or cervical cancer AND at least 1 of HPV types 35, 39, 51, 56, or 59 is detected by Thinsection PCR in an adjacent section from the same tissue block.

2. *CIN (any grade), AIS, cervical cancer, vulvar cancer or vaginal cancer related to HPV 6, 11, 16, 18, 31, 33, 45, 52, or 58*. This endpoint is defined to have occurred if on a single biopsy or excised tissue, there is the NPP consensus diagnosis of CIN 1, CIN 2, CIN 3, AIS, cervical cancer, vulvar cancer or vaginal cancer AND at least 1 of HPV types 6, 11, 16, 18, 31, 33, 45, 52, or 58 is detected by Thinsection PCR in an adjacent section from the same tissue block.

The exploratory effectiveness endpoints are as follows:

1. Incidence of CIN 2 or 3, AIS, or cervical cancer, as defined in the primary endpoint, but irrespective of HPV-relatedness.

2. Incidence of CIN (any grade), AIS, cervical cancer, vulvar cancer, or vaginal cancer, as defined in the secondary endpoint, but irrespective of HPV-relatedness.

3. Incidence of Pap abnormalities.

4. Incidence of HPV positivity, data permitting.

5. Incidence of CIN 2, CIN 3, AIS, and cervical cancer related to HPV 31, 33, 45, 52, and 58 in women who are vaccinated with GARDASIL™ and then the 9vHPVvaccine in the base study, data permitting.

3.5.3.2 Immunogenicity

The immunogenicity endpoints are GMTs and seropositivity rates to HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58. A subject with a cLIA or IgG titer at or above the serostatus cutoff for a given HPV type is considered seropositive for that type. In GMT and seropositivity analyses, each vaccine component (i.e., HPV type) will be analyzed separately.

3.5.3.3 Safety

Information on deaths, cancers, hospitalizations, and other safety outcomes will be collected for safety assessment. These data will be collected through registry searches performed by the NRSCs.

3.5.4 Analysis Populations

3.5.4.1 Effectiveness

Per-Protocol Analysis

The primary approach for all effectiveness analyses will use the PPE population. For Cohort 1, subjects will be included in the PPE analysis as defined in the original and subsequent analyses of Protocol V503-001, i.e., if they were seronegative and PCR-negative at baseline and PCR-negative through Month 7 to the appropriate HPV type(s), received 3 doses of the 9vHPVvaccine within 1 year, did not violate the protocol, and have any follow-up visit in the LTFU study. A PPE population cannot be defined for Cohort 2, due to the Month 7 PCR criterion and because this cohort will not have had the same vaccine regimen as Cohort 1. Therefore, per-protocol analyses will not be performed for Cohort 2.

Modified Intent-to-Treat Analysis

A supportive effectiveness analysis approach will be modified intention-to-treat (MITT). Subjects who received at least 1 vaccination, have any follow-up visit in the LTFU study, and were seronegative (by cLIA) and PCR-negative to the appropriate HPV type(s) prior to vaccination will be included. This population is referred to as the HPV Type-Specific Naïve (HN-TS) population.

Any analyses of disease irrespective of HPV type-relatedness will use a population similar to the MITT population. In this population, only subjects who are seronegative and PCR-negative to all HPV types tested and have a Pap test negative for squamous intraepithelial lesion (SIL) prior to vaccination will be eligible to be counted as cases. This population is referred to as the All HPV Naïve (All-HN) population.

For Cohort 1, the MITT populations will be based on Pap, PCR- and sero-status at the time of first vaccination with the 9vHPVvaccine, i.e., Day 1 of the base study. If analysis of Cohort 2 is performed, the MITT populations will be based on Pap, PCR- and sero-status throughout the base study.

If subjects who initially received an incomplete regimen of the 9vHPVvaccine receive catch-up vaccination within a protocol amendment, they will be included in Cohort 1 by virtue of their original randomization, although they will only be included in MITT analyses.

All Subjects As-Randomized

A further supportive analysis population will be all subjects as randomized. This analysis includes all subjects who received at least one vaccination and provided follow-up, regardless of their serology or PCR status prior to vaccination. This population is referred to as the Full Analysis Set (FAS).

3.5.4.2 Immunogenicity

The primary approach to the analyses of immunogenicity will be the per-protocol immunogenicity (PPI) approach. For Cohort 1, subjects will be included in the PPI analysis as defined in the original and subsequent analyses of Protocol V503-001, i.e., if they were seronegative (by cLIA) and PCR-negative at baseline and PCR-negative through Month 7 to the appropriate HPV type(s), received 3 doses of the 9vHPVvaccine within acceptable windows, and did not violate the protocol. A PPI population cannot be defined for Cohort 2, due to the Month 7 PCR criterion and because this cohort will not have had the same vaccine regimen as Cohort 1. Therefore, a per-protocol analysis will not be performed for Cohort 2.

3.5.4.3 Safety

All subjects who received at least one dose of the 9vHPVvaccine or the GARDASIL™ and have follow-up data will be included in the analysis of safety in this study.

3.5.5 Statistical Methods

3.5.5.1 Effectiveness

Table 3-1 summarizes the planned analyses of effectiveness. Analyses of 9vHPV vaccine effectiveness will be based on Cohort 1. Data from Cohort 2 may be used for exploratory analyses.

Table 3-1

Analysis Strategy for Effectiveness Endpoints

Endpoint/Variable	Primary vs. Supportive Approach[†]	Statistical Method[‡]	Analysis Population
Primary Objective/Hypotheses – Prophylactic Effectiveness			
HPV 16/18/31/33/45/52/58-related CIN grades 2 or 3, AIS, or cervical cancer (Overall, by time since vaccination, by HPV type, and by lesion type)	P	Control Chart Analysis	PPE – Cohort 1
	P	Analysis of Vaccine Effectiveness	PPE – Cohort 1
	S	Control Chart Analysis	HN-TS – Cohort 1 FAS – Cohort 1
	S	Analysis of Vaccine Effectiveness	HN-TS – Cohorts 1 FAS – Cohorts 1
	S	Estimation of Incidence Rates	HN-TS – Cohort 2 FAS – Cohort 2
	S	Kaplan-Meier Plot[‖]	PPE – Cohort 1 HN-TS – Cohorts 1 & 2 FAS – Cohorts 1 & 2
HPV 16/18/31/33/45/52/58-related CIN grades 2 or 3, AIS, or cervical cancer (By baseline characteristics[§])	S	Analysis of Vaccine Effectiveness	PPE – Cohort 1
HPV 16/18/31/33/45/52/58-related CIN grades 2 or 3, AIS, or cervical cancer (By local pathology diagnosis[¶])	S	Analysis of Vaccine Effectiveness	PPE – Cohort 1
Secondary Objective 1 – Prophylactic Effectiveness			
HPV 35/39/51/56/59-related CIN grades 2 or 3, AIS, or cervical cancer (Overall, by time since vaccination, by HPV type, and by lesion type)	P	Estimation of Incidence Rates	HN-TS – Cohorts 1
	S	Estimation of Incidence Rates	HN-TS – Cohort 2
	S	Estimation of Incidence Rates	FAS – Cohorts 1 & 2
	S	Kaplan-Meier Plot[‖]	HN-TS – Cohorts 1 & 2 FAS – Cohorts 1 & 2
Secondary Objective 2 – Prophylactic Effectiveness			
HPV 6/11/16/18/31/33/45/52/58-related CIN (any grade), AIS, cervical cancer, vulvar cancer, or vaginal cancer (Overall, by time since vaccination, by HPV type, and by lesion type)	P	Estimation of Incidence Rates	PPE – Cohort 1
	S	Estimation of Incidence Rates	HN-TS – Cohorts 1 & 2 FAS – Cohorts 1 & 2
	S	Kaplan-Meier Plot[‖]	PPE – Cohort 1 HN-TS – Cohorts 1 & 2 FAS – Cohorts 1 & 2

Analysis Strategy for Effectiveness Endpoints (Cont.)

Endpoint/Variable	Primary vs. Supportive Approach[†]	Statistical Method[‡]	Analysis Population
Other Exploratory Endpoints – Population Benefit			
CIN grades 2 or 3, AIS, or cervical cancer irrespective of HPV type (Overall and by HPV type)	S	Estimation of Incidence Rates	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2
	S	Kaplan-Meier Plot[‖]	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2
CIN (any grade), AIS, cervical cancer, vulvar cancer, or vaginal cancer irrespective of HPV type (Overall and by HPV type)	S	Estimation of Incidence Rates	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2
	S	Kaplan-Meier Plot[‖]	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2
Pap test abnormalities (Overall and by severity)	S	Estimation of Incidence Rates	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2
	S	Kaplan-Meier Plot[‖]	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2
HPV positivity (Data permitting)	S	Estimation of Incidence Rates	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2
	S	Kaplan-Meier Plot[‖]	All-HN – Cohorts 1 & 2 FAS – Cohorts 1 & 2

[†] P = Primary approach; S = Supportive approach. Supportive analyses for Cohort 2 may be conducted and are considered exploratory.
[‡] Statistical methods are described in further detail below.
[§] Baseline characteristics include age, country, lifetime number of sexual partners at enrollment, number of new sexual partners in 6 months prior to enrollment, prevalence of sexually transmitted disease, pregnancy history, Day 1 pap status, Day 1 PCR status, and Day 1 serostatus as defined in the original V503-001 study.
[‖] A Kaplan-Meier curve will be produced when at least five cases of the effectiveness endpoint have occurred.
[¶] The local pathology diagnosis is the diagnosis given by the local pathologist for patient care.

At each analysis, incidence for each effectiveness endpoint will be estimated by year following vaccination (by cohort) and cumulatively [4]. Incidence estimates and 95% CIs will be provided based on (i) total follow-up time within the LTFU study and (ii) total follow-up time from the beginning of the base study (Cohort 1 only).

For the primary endpoint of HPV 16/18/31/33/45/52/58-related CIN 2 or worse, effectiveness will be summarized relative to defined comparison datasets; primarily, relative to the cohort with similar numbers of sexual partners as identified by the CCS survey and described in Table 3-2; but also to the full population incidence data from the NRSCs as available, with and without a correction for the frequency of screening. All breakthrough cases in the primary effectiveness analysis, regardless of number or time of occurrence, will be investigated and case narratives will be included in any reports or summaries.

For the secondary effectiveness and population benefit endpoints, point estimates and 95% CIs of cumulative incidence will be provided. It is expected that not all low-grade

cervical lesions will be biopsied within the NRSC system, so there will be incomplete ascertainment of CIN 1. For this reason, there will be no estimation of vaccine effectiveness for this endpoint.

Standards of care are changing and the Nordic countries may begin conducting HPV tests along with Pap smears in the future. If data are available for HPV tests within the registries, HPV positivity will be summarized similarly to Pap test abnormalities.

Cohort 2 will consist of subjects who have either received GARDASIL™ only and/or subjects who received GARDASIL™ followed by the 9vHPVvaccine, depending on whether the subjects in the Protocol V503-001 base study opt to receive 3 doses of 9vHPVvaccine as part of a study extension. If Cohort 2 is analyzed for exploratory analyses, the subgroups of Cohort 2 will be analyzed separately if data are available in each subgroup. The vaccine doses received will need to be considered when interpreting the efficacy analyses for this cohort. Subjects in Cohort 2 vaccinated with the 9vHPV vaccine will have approximately 5 more years of potential exposure than subjects in Cohort 1 to HPV types 31, 33, 45, 52, and 58 prior to vaccination, and their HPV exposure prior to vaccination may not be fully understood. Therefore, results from Cohort 2 effectiveness analyses may be difficult to interpret.

Control Chart Method for Monitoring Vaccine Effectiveness

Establishment of Control Rates

In the absence of a concurrent control group, incidence rates observed in the LTFU study must be compared with other data sources. Extensive efforts have been made to identify the most appropriate source of information on the expected number of disease cases which might have occurred in an unvaccinated cohort.

Information from the placebo groups in studies of GARDASIL™ could be used to provide comprehensive disease incidence data among unvaccinated Nordic region subjects, including full HPV-relatedness data. However, it is recognized that the intensity of follow-up required by the clinical protocols leads to greater ascertainment of disease than would occur in registry-based studies. Even within the Nordic registry setting, more aggressive follow-up strategies have led to higher disease ascertainment [5].

Information from the Nordic registries prior to the introduction of GARDASIL™ accurately measures the incidence of disease in the entire population in an unvaccinated state, within those countries. However, surveys of sexual activity in the region [1] indicate that the level of exposure to HPV disease in the general population is higher than is expected in the Protocol V503-001 study population. For the age range 18 to 23 years, the median number of lifetime sexual partners in the general population is 4. An inclusion criterion of the main study was to have 4 or fewer lifetime partners, and so the median number of partners among subjects enrolled within the Nordic region is less than 4. Therefore, an expected number of cases based on the entire Nordic population data may also be too high.

It has been possible to identify subjects with lower numbers of sexual partners from the above-mentioned survey (referred to as the CCS) and track their disease incidence within the registries. Incidence of CIN 2 or worse among subjects 23 to 29 years old with 1 to 6 sexual partners is as shown in Table 3-2. This is the approximate age and sexual activity profile expected of the Protocol V503-001 Nordic population at the time the LTFU study begins.

Table 3-2

Incidence of CIN 2 or Worse Among Female Subjects 23 to 29 Years of Age in The CCS Cohort by Lifetime Number of Sexual Partners (January 2004 to August 2006)

Country	# Lifetime Sexual Partners	CIN 2+ incidence / 1000 Person-Years[†] (95% CI)	Approximate % of Subjects in LTFU
Denmark	1 to 6 Any	5.9 (3.7, 8.0) 11.2 (9.1, 13.3)	82%
Norway	1 to 6 Any	2.8 (1.5, 4.9) 5.9 (4.3, 7.8)	15%
Sweden	1 to 6 Any	7.3 (3.3, 13.9) 9.3 (5.8, 14.0)	3%

[†] Incidence among subjects with Pap smears within the observation interval.

These data cover a period prior to availability of GARDASIL™, so they represent an estimate of incidence in an unvaccinated population. Combining the above country-specific rates in proportion to their contribution to the LTFU study, the assumed rate of all CIN 2 or worse in the LTFU cohort, had they remained unvaccinated, will be 5.48/1000 person-years.

Over the course of the LTFU, the subjects will get older and their risk profile will change. Similar data to that displayed in Table 3-1 were obtained in older 30 to 36 years of age CCS subjects, approximating the age of the cohort at the end of the LTFU study in 2025. The rate of all CIN 2 or worse in this group was similar to that found in 23- to 29 year-olds. Thus, as an approximation, the background rate will be assumed constant at 5.48/1000 person-years.

Finally, since the registry data do not include HPV type-relatedness, a fraction of this disease incidence is assumed to be related to HPV 16/18/31/33/45/52/58 for the breakthrough analysis. Recent literature indicates that HPV 16/18/31/33/45/52/58 are present in 75 to 85% of cervical lesions with a diagnosis of CIN 2/3 [6,7,8,9,10], 95% of cervical lesions with a diagnosis of AIS [6,7,10,11,12,13], and 90% of cervical lesions with a diagnosis of cancer [14], worldwide. Based on the Vaccine Impact in Population (VIP) study [15], HPV 16/18/31/33/45/52/58 were present in 72 to 77% of cervical lesions with a diagnosis of CIN 2; 83% of cervical lesions with a diagnosis of CIN 3, and 90 to 92% of cervical lesions with a diagnosis of cancer, in Sweden. Due to the

percentages of lesions expected with these diagnoses in the Protocol V503-001 LTFU study, it will be assumed that 80% of the CIN 2/3, AIS, or cancer lesions in the population data are related to HPV 16/18/31/33/45/52/58 (i.e., absolute incidence rate of 4.38/1000 person-years).

If additional data become available prior to the first effectiveness analysis which would change the proposed reference rates, updated information will be described in the study report. No changes to the reference rates will be made after the first effectiveness analysis.

Breakthrough Disease

A control chart, or threshold monitoring approach, for breakthrough disease incidence will be used to guide decision-making within the LTFU study. For the primary analysis in the PPE population, the control chart is designed to indicate when the breakthrough rate is exceeding 10% of the estimated reference incidence rate in unvaccinated subjects (in other words, when vaccine effectiveness is decreasing to less than 90%). In absolute terms, 10% of the target rate is 0.438/1000 person-years. The observed breakthrough rate will be monitored by use of an adapted Shewhart *c*-chart, which is described in Section 6.2. A 2.75-sigma control limit above the center line, i.e., target rate, will be used. In addition, a 1.83-sigma control limit will be used to incorporate a 2-out-of-3 runs rule for increased sensitivity to detect small shifts in vaccine effectiveness.

For each analysis, the observed cases of breakthrough disease (HPV 16/18/31/33/45/52/58-related CIN 2/3, AIS, or cervical cancer) will be plotted in time intervals based on time since vaccination and compared to the control limits to determine if a signal is produced. Exact control limits will depend upon the actual years of follow-up accrued at an analysis. The time intervals in the control chart will begin at Year 4 (i.e., Month 48), which corresponds to 3.417 years following the Month 7 visit for the PPE population and 4 years following the first vaccination for the HNRT and FAS populations. Four years was selected as the starting point for the analysis since this is expected to be the average length of time subjects are enrolled in the main study. The time intervals will have a width of 2 years, based on the screening schedule anticipated in the Nordic countries. For each time interval in the control chart, the number of cases will be plotted only when there has been enough follow-up time accrued in the interval to adequately assess disease incidence. If an interval has at least 60% of the total expected person-years of follow-up time accrued or if it is clear a signal will be detected when at least 60% of the total expected follow-up time is accrued, a point will be estimated and plotted. Figures 6-1 through 6-6 in Section 6.2 provide examples of the control limits to be applied at each analysis in the LTFU study. These control limits were produced based on the target incidence rate and simulated visit data over the 10-year follow-up period for the primary effectiveness endpoint in the PPE population.

The signaling rule for opening substantive discussions with regulatory agencies on actions to be taken with regard to waning effectiveness is (1) if the number of cases exceeds the 2.75-sigma control limit once in any analysis, or (2) if the number of cases

exceeds the 1.83-sigma control limit on 2 occasions over 3 consecutive intervals in any analysis. As an illustration of the signaling rule, if discontinuation is low and subjects adhere to the 3-year screening recommendations, this signaling rule will be equivalent to signaling if 5 cases are observed in any 2-year interval, or if 4 cases are observed in 2 out of 3 consecutive 2-year intervals. If a signal is detected, the data analyses from this study will be interpreted in the context of other long-term follow-up studies being conducted for 9vHPVvaccine. In addition, an international panel of experts in the fields of epidemiology, vaccine usage and effectiveness, and cost/benefit analysis will be convened to review the data and advise the Sponsor on the need for a booster. It will review individual breakthrough cases to determine if those cases represent host-specific conditions that might explain the waning immunity or if the cases represent normal hosts with waning vaccine effect. Narratives will be written for each breakthrough case.

It is important to consider what the estimated vaccine effectiveness might be after completion of the LTFU study, and its associated variability. Table 3-3 provides an example of a pattern of breakthrough cases over the course of the LTFU study and the estimates and 95% for vaccine effectiveness, given for the current analysis period and on a cumulative basis since the start of the LTFU. This example corresponds to the example shown in Section 6.2.4 starting at Year 4.

Table 3-3

Estimates of Current and Cumulative Vaccine Effectiveness
After an Example Pattern of Breakthrough Cases

Analysis	Person-Years Accrued	Breakthrough Cases	Current Breakthrough Rate as % VE (95% CI)		Cumulative Breakthrough Rate as % VE (95% CI)	
1	864	1	74%	(-47%,99%)	74%	(-47%,99%)
2	3,375	2	86%	(51%, 98%)	84%	(53%, 97%)
3	3,175	5	64%	(16%, 88%)	75%	(51%, 89%)
4	3,277	3	79%	(39%, 96%)	77%	(58%, 88%)
5	3,286	3	79%	(39%, 96%)	77%	(62%, 87%)
In an unvaccinated cohort, approximately 4 cases would be expected in the first two year analysis period and approximately 14 cases would be expected in all other 2 year analysis periods, assuming a background incidence of 4.38 / 1,000 person-years.						

In addition to the signaling rule based on the control chart analysis, it is acknowledged that other patterns of breakthrough cases may raise concern over waning effectiveness. For example, a gradually increasing pattern of breakthroughs over several analyses would lead to concern that effectiveness was waning, even if the rate of breakthroughs was still below the threshold. Thus, the formal control chart signaling rule will be augmented with an additional condition which will prompt discussions with regulatory agencies: if cumulative breakthroughs over 2 or more analyses since the start of the LTFU study are such that the estimated cumulative vaccine effectiveness has a 95% CI with an upper

bound less than 90%. Note that in the example shown in Table 3-3, a signal would be produced based on cumulative vaccine effectiveness at Analysis 4.

Analysis of Vaccine Effectiveness

As part of the primary effectiveness monitoring plan the cumulative vaccine effectiveness and the corresponding 95% CI will be calculated to determine if the upper bound is below 90%. Although a formal hypothesis test will not be conducted, the comparison can be thought of in terms of the following hypothesis:

$$H_0: \lambda \geq 0.9 \text{ vs. } H_1: \lambda < 0.9$$

where λ is cumulative vaccine effectiveness, the relative risk reduction on vaccine compared to an unvaccinated cohort, based on the cumulative follow-up time accrued when the analysis is performed. λ may be written $\lambda = 1-(r_v/r_u)$ where r_v, the incidence rate among vaccine recipients, is defined as $r_v = C_v/\tau_v$, C_v = the number of primary effectiveness cases among vaccine recipients and τ_v = the total person-years of follow-up among vaccine recipients, and r_u is the incidence rate among an unvaccinated cohort.

Under the assumption that r_v is the mean of an independent Poisson process, the number of primary effectiveness cases C_v among vaccine recipients is distributed as Poisson(r_v). An exact 100(1-α)% confidence interval for r_v can be obtained by calculating

$$LB(r_v) = 0.5\{\chi^2_{df=2Cv,\ \alpha/2}\},$$

the lower bound, and

$$UB(r_v) = 0.5\{\chi^2_{df=2(Cv+1),\ 1-\alpha/2}\},$$

the upper bound, where $\chi^2_{df=2Cv,\ \alpha/2}$ is the (100) α/2 percentile of the chi-square distribution with $2C_v$ degrees of freedom and $\chi^2_{df=2(Cv+1),\ 1-\alpha/2}$ is the (100) (1-α/2) percentile of the chi-square distribution with $2(C_v+1)$ degrees of freedom.

The exact 100(1-α)% CI for cumulative vaccine effectiveness will then have a lower bound equal to

$$VE_L = 100\%*\{1 - (UB(r_v)/\ \tau_v)/\ r_u\}$$

and an upper bound equal to

$$VE_U = 100\%*\{1 - (LB(r_v)/\ \tau_v)/\ r_u\}.$$

Computation of Follow-Up Time

Follow-up for the primary control chart analysis in the PPE population begins 3.417 years following the Month 7 visit. Therefore, the follow-up time for a subject will be the interval in years between Year 4 or the time the subject exits the base study, whichever is

later, and her last day of follow-up in the Nordic LTFU study. For cases, the last day of follow-up will be the visit at which the biopsy detecting the endpoint was taken. If a subject develops more than one case of disease that fits into a given endpoint classification, the final visit date will be the date at which the first of these endpoints was detected. For non-cases, the last day of follow-up will be defined as the date of the last pap test. For the MITT and FAS analyses, follow-up begins 4 years following the time of first vaccination with the 9vHPVvaccine (i.e., Day 1), so the follow-up time for a subject will be the interval in years between Year 4 and the last day of follow-up, as described above.

For other effectiveness and incidence analyses, follow-up time begins at the end of the base study for those who received V503 at the start of the base study. For those who received GARDASIL™ at the beginning of the base study, the follow-up time calculations will depend on whether or not they receive V503 at the end of the base study. If V503 is offered, then subjects who receive it will be in a new cohort and their follow-up time will start when they are vaccinated. For those subjects who only receive GARDASIL™, their follow-up time will be calculated in the same way as those who were originally given V503. For cases, the last day of follow-up will be the visit at which the biopsy detecting the endpoint was taken. If a subject develops more than one case of disease that fits into a given endpoint classification, the final visit date will be the date at which the first of these endpoints was detected. For non-cases, the last day of follow-up will be defined as the date of the last Pap test.

The date of occurrence of each endpoint is defined as the date of the sample from which the diagnosis of disease was made.

Counting Endpoint Cases

For the effectiveness analyses, if a subject has experienced one *or more* of the components of a composite endpoint, she will be classified as a single case for the analysis at the time of detection of the first endpoint. For example, with respect to the primary endpoint, a subject may have had AIS related to HPV 16 and CIN 2 related to HPV 18, but she will count as a single case toward the primary analysis. For summaries in which cases of the composite endpoint are further classified by component, such as by lesion type or HPV type, a subject will be counted as a case at most once in each applicable sub-category but may appear in multiple sub-categories. In the example above, the subject would be counted as a case in the summary of HPV 16-related disease, and also in the summary of HPV 18-related disease.

Kaplan-Meier Estimation

For effectiveness endpoints where at least five cases have occurred during the extension period, a Kaplan-Meier curve will be produced to illustrate accrual of cases over time since vaccination with the 9vHPVvaccine. These curves will be produced separately for Cohorts 1 and 2 (if either group meets the 5-case criterion).

Missing Data

There will be no imputation of results where data are missing. Biopsy samples with missing PCR results cannot be counted as a case for the primary analysis, but will be included in the exploratory analyses of cases irrespective of HPV type. An NPP consensus diagnosis is required to be a case for the primary analysis.

3.5.5.2 Immunogenicity

Table 3-4 summarizes the planned analyses of immunogenicity.

Table 3-4

Analysis Strategy for Immunogenicity Endpoints

Endpoint/Variable	Primary vs. Supportive Approach[†]	Statistical Method[‡]	Analysis Population
Secondary Objective – Immunogenicity Responses			
Anti-HPV responses for HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 (By number of years since first vaccination[§])	P	GMTs	PPI – Cohort 1
	S	Longitudinal Plots of GMTs[‖]	PPI – Cohort 1
	S	RCD Plots	PPI – Cohort 1
Seropositivity for HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 (By number of years since first vaccination)	P	Seropositivity Percentages	PPI – Cohort 1

[†] P = Primary approach; S = Supportive approach.
[‡] Statistical methods are described in further detail below.
[§] Immunogenicity data collected during the LTFU study will be summarized over yearly intervals of time since the day of first vaccination.
[‖] Longitudinal plots will include results from V503-001 in addition to results from the LTFU study.
GMTs = Geometric mean titers; RCD = Reverse cumulative distribution.

The time points at which antibody data will be collected are approximately 5 years and 10 years after the start of the LTFU study, i.e., approximately 9 years and 14 years after Cohort 1 receives their first dose of the 9vHPVvaccine. Anti-HPV responses for each of the 9 vaccine HPV types will be summarized in terms of GMTs with 95% CI based on the time since the first vaccination with the 9vHPVvaccine. These CIs will be constructed based on the assumption that log titers follow a normal distribution. Anti-HPV responses will be investigated using longitudinal plots of the GMTs, incorporating results from the main study follow-up period, relative to day of first vaccination. Reverse cumulative distribution (RCD) plots and a summary of seropositivity percentages, including 95% CIs, will be presented for each of the 9 vaccine HPV types based on the time since the first vaccination with the 9vHPVvaccine. RCD plots may also be used to compare the all HPV-naïve subjects population with subsets of interest (e.g. subjects who had HPV-related disease during the base study follow-up). In all analyses, responses will be summarized over yearly intervals of time since the day of first vaccination.

The minimum protective antibody level will be investigated by comparing antibody responses and risk of disease. In Cohort 1, Month 7 anti-HPV (cLIA/IgG) levels will be observationally compared between subjects who became cases of disease (at any time after Month 7) and subjects who remained disease-free (data permitting). Additional analyses will examine anti-HPV levels observed at the most recent time point prior to becoming a breakthrough case.

3.5.5.3 Safety

The health outcome data collected by the NRSCs related to deaths, cancers, hospitalizations, and other safety outcomes will be used to measure long-term safety in subjects vaccinated with the 9vHPVvaccine. The rates of these outcomes in the LTFU study may be compared to published rates in the age and sex-matched general population.

3.5.6 Multiplicity Considerations

Since formal hypothesis testing will not be carried out, multiplicity adjustment is not relevant. There will be repeated analyses of the effectiveness endpoints during the lifetime of the LTFU study. The assessment of the type I error rate and power for the proposed control limits take into account that multiple analyses will be performed.

3.5.7 Sample Size and Power Calculations

Effectiveness

The sample size for the LTFU study is fixed by the number of Protocol V503-001 base study participants who are eligible and willing to participate in the LTFU extension. Because no hypotheses will be tested, the concept of power is not completely relevant. However, it is important to consider the performance of the control chart established in Section 3.5.5.1, in the presence of various waning effectiveness models.

There are approximately 4453 subjects total in Cohorts 1 and 2. Only those subjects in Cohort 1 are considered in the performance assessment of the control chart, since these subjects will contribute to the primary effectiveness analysis in the PPE population. Approximately 1900 subjects in Cohort 1 are expected to be eligible for the LTFU primary effectiveness analysis in the PPE population based on attrition during Protocol V503-001 primary follow-up, ineligibility for the analysis population, or unwillingness to consent to LTFU.

To evaluate the adapted Shewhart *c*-chart in the context of the Nordic LTFU study, simulations were performed. In the LTFU study, subjects are expected to attend screening visits approximately 3 years apart over the 10-year follow-up period. Detailed information has been provided by the Nordic Cancer Registries to accurately simulate subject visit schedules and the outcomes for each visit over the 10 years of follow-up. Using this information, along with the target rate established for the primary effectiveness analysis in the PPE population, visit outcomes were simulated based on different underlying vaccine effectiveness models. These outcomes were analyzed using

the control chart method with different control limit settings to assess the alpha-level of the control chart.

To control the alpha-level, or type I error rate, of the control chart, visit data were simulated under the assumption that vaccine effectiveness is 90% over the entire 10-year follow-up period. These data were analyzed using the adapted Shewhart *c*-chart with various control limits and signaling rules. If a signaling rule is adopted where the chart signals when (1) the number of cases exceeds the 2.75-sigma control limit once in any analysis, or (2) if the number of cases exceeds the 1.83-sigma control limit on 2 occasions over 3 consecutive intervals in any analysis, then the approximate alpha-level of the control chart is 0.078 (approximate 95% CI: 0.062, 0.094). The 95% CI is based on the normal approximation to the binomial distribution. This signaling rule was chosen for use in the LTFU study because it gives an alpha-level of approximately 0.05.

To determine the power of the control chart, simulations were performed applying the signaling rule above under 4 different vaccine waning scenarios. These scenarios included:

1. A linear decrease in vaccine effectiveness from 90% to 50% over the 10-year follow-up period.

2. A linear decrease in vaccine effectiveness from 90% to 70% over the 10-year follow-up period.

3. Constant vaccine effectiveness of 70% over the 10-year follow-up period.

4. Constant vaccine effectiveness of 80% over the 10-year follow-up period.

Scenarios 1 and 3 have an average vaccine effectiveness of 70% over the 10-year follow-up period. Likewise, scenarios 2 and 4 have an average vaccine effectiveness of 80% over the 10-year follow-up period. Table 3-5 provides the power estimates for each of these scenarios.

Table 3-5

Power of the Shewhart *c*-Chart
For Given True Vaccine Effectiveness Models

Vaccine Effectiveness (VE) Waning Model	Average Vaccine Effectiveness Over Follow-Up Period	Power Estimate	95% Confidence Interval[†]
(1) Linear decrease from 90% VE to 50%	70%	95.2%	(93.9%, 96.5%)
(2) Linear decrease from 90% VE to 70%	80%	62.2%	(59.2%, 65.2%)
(3) Constant VE at 70%	70%	95.8%	(94.6%, 97.0%)
(4) Constant VE at 80%	80%	61.3%	(58.3%, 64.3%)
[†]Confidence intervals are based on the normal approximation to the binomial distribution.			

In scenario 1, there is approximately 95.2% (approximate 95% CI: 93.9, 96.5) power to detect the shift in vaccine effectiveness. The other scenarios show that over the 10-year follow-up period, the power decreases for shifts in vaccine effectiveness that remain closer to 90%.

3.5.8 Interim Analyses

There will be multiple analyses of effectiveness data during the LTFU study. The frequency of analyses may change (increase) depending on results previously observed.

3.5.9 Definition of Compliance Measure

Vaccine-related compliance is not relevant to this protocol because no vaccinations are provided within it. However, the numbers of subjects who received vaccinations in the original study will be tabulated, by cohort.

An important compliance measure with respect to the LTFU study is the degree of adherence to national recommendations for screening examinations for participants of the LTFU study. The numbers of subjects who have attended Pap screening visits at each analysis will be summarized and compared with the expected numbers assuming full compliance with the recommendations. Frequency of Pap screening visits will be summarized overall and by country. The potential impact on the analyses of effectiveness will be discussed.

3.6 DATA MANAGEMENT

Information regarding Data Management procedures for this protocol will be provided by the SPONSOR.

3.7 BIOLOGICAL SPECIMENS

Information regarding biological specimens and sample labeling for this protocol will be provided in the Administrative Binder.

4. ADMINISTRATIVE AND REGULATORY DETAILS

4.1 CONFIDENTIALITY

4.1.1 Confidentiality of Data

For Studies Conducted Under the U.S. IND

Particular attention is drawn to the regulations promulgated by the Food and Drug Administration under the Freedom of Information Act providing, in part, that information furnished to clinical investigators and Institutional Review Boards will be kept confidential by the Food and Drug Administration only if maintained in confidence by the clinical investigator and Institutional Review Board.

For All Studies

By signing this protocol, the investigator affirms to the SPONSOR that information furnished to the investigator by the SPONSOR will be maintained in confidence and such information will be divulged to the Institutional Review Board, Ethics Review Committee, or similar or expert committee; affiliated institution; and employees only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this study will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

4.1.2 Confidentiality of Subject/Patient Records

For All Studies

By signing this protocol, the investigator agrees that the SPONSOR (or SPONSOR representative), Institutional Review Board/Independent Ethics Committee (IRB/IEC), or Regulatory Agency representatives may consult and/or copy study documents in order to verify worksheet/case report form data. By signing the consent form, the subject/patient agrees to this process. If study documents will be photocopied during the process of verifying worksheet/case report form information, the subject/patient will be identified by unique code only; full names/initials will be masked prior to transmission to the SPONSOR.

For Studies Conducted Under the U.S. IND

By signing this protocol, the investigator agrees to treat all patient data used and disclosed in connection with this study in accordance with all applicable privacy laws, rules and regulations, including all applicable provisions of the Health Insurance Portability and Accountability Act and its implementing regulations, as amended from time to time ("HIPAA").

4.1.3 Confidentiality of Investigator Information

For All Studies

By signing this protocol, the investigator recognizes that certain personal identifying information with respect to the investigator, and all subinvestigators and study site

personnel, may be used and disclosed for study management purposes, as part of a regulatory submissions, and as required by law. This information may include:

- name, address, telephone number, and email address;
- hospital or clinic address and telephone number;
- curriculum vitae or other summary of qualifications and credentials; and
- other professional documentation.

Consistent with the purposes described above, this information may be transmitted to the SPONSOR, and subsidiaries, affiliates and agents of the SPONSOR, in your country and other countries, including countries that do not have laws protecting such information. Additionally, the investigator's name and business contact information may be included when reporting certain serious adverse events to regulatory agencies or to other investigators. By signing this protocol, the investigator expressly consents to these uses and disclosures.

For Multicenter Studies
In order to facilitate contact between investigators, the SPONSOR may share an investigator's name and contact information with other participating investigators upon request.

4.2 COMPLIANCE WITH LAW, AUDIT, AND DEBARMENT

By signing this protocol, the investigator agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice; and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

The Code of Conduct, a collection of goals and considerations that govern the ethical and scientific conduct of clinical investigations sponsored by Merck, is attached.

The investigator also agrees to allow monitoring, audits, Institutional Review Board/ Independent Ethics Committee review, and regulatory agency inspection of trial-related documents and procedures and provide for direct access to all study-related source data and documents.

The investigator agrees not to seek reimbursement from subjects/patients, their insurance providers, or from government programs for procedures included as part of the study reimbursed to the investigator by the SPONSOR.

The Investigator shall prepare and maintain complete and accurate study documentation in compliance with Good Clinical Practice standards and applicable federal, state, and local laws, rules and regulations; and, for each subject/patient participating in the study, provide all data, and upon completion or termination of the clinical study submit any

other reports to the SPONSOR as required by this protocol or as otherwise required pursuant to any agreement with the SPONSOR.

Study documentation will be promptly and fully disclosed to the SPONSOR by the investigator upon request and also shall be made available at the investigator's site upon request for inspection, copying, review, and audit at reasonable times by representatives of the SPONSOR or any regulatory agencies. The investigator agrees to promptly take any reasonable steps that are requested by the SPONSOR as a result of an audit to cure deficiencies in the study documentation and worksheets/case report forms.

International Conference of Harmonization Good Clinical Practice guidelines (Section 4.3.3) recommend that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

According to European legislation, a SPONSOR must designate a principal or coordinating investigator (CI) to review the report (summarizing the study results) and confirm that to the best of his/her knowledge the report accurately describes conduct and results of the study. The SPONSOR may consider one or more factors in the selection of the individual to serve as the CI (e.g., thorough understanding of clinical trial methods, appropriate enrollment of subject/patient cohort, timely achievement of study milestones, availability of the CI during the anticipated review process).

The investigator will promptly inform the SPONSOR of any regulatory agency inspection conducted for this study.

Persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on this SPONSOR's studies. The investigator will immediately disclose in writing to the SPONSOR if any person who is involved in conducting the study is debarred, or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened.

In the event the SPONSOR prematurely terminates a particular trial site, the SPONSOR will promptly notify that site's IRB/IEC.

4.3 COMPLIANCE WITH FINANCIAL DISCLOSURE REQUIREMENTS

By signing this protocol, the investigator agrees to provide to the SPONSOR accurate financial information to allow the SPONSOR to submit complete and accurate certification and disclosure statements as required by U.S. Food and Drug Administration regulations (21 CFR Part 54). The investigator further agrees to provide this information on a Financial Disclosure/Certification Form that is provided by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. This requirement also extends to subinvestigators. The investigator also consents to the transmission of this information to Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. in the United States for these purposes. This may involve the transmission of information to countries that do not have laws protecting personal data.

4.4 QUALITY CONTROL AND QUALITY ASSURANCE

By signing this protocol, the SPONSOR agrees to be responsible for implementing and maintaining quality control and quality assurance systems with written SOPs to ensure that trials are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

4.5 COMPLIANCE WITH INFORMATION PROGRAM ON CLINICAL TRIALS FOR SERIOUS OR LIFE THREATENING CONDITIONS

Under the terms of The Food and Drug Administration Modernization Act (FDAMA), the SPONSOR of the study is solely responsible for determining whether the study is subject to the requirements for submission to the Clinical Trials Data Bank, http://clinicaltrials.gov/. Merck, as SPONSOR of this study, will review this protocol and submit the information necessary to fulfill this requirement. Merck entries are not limited to FDAMA mandated trials. Merck's voluntary listings, beyond those mandated by FDAMA, will be in the same format as for treatments for serious or life-threatening illnesses. Information posted will allow patients to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate study locations and site contact information.

By signing this protocol, the investigator acknowledges that the statutory obligation under FDAMA is that of the SPONSOR and agrees not to submit any information about this study to the Clinical Trials Data Bank.

4.6 PUBLICATIONS

As this study is part of a multicenter trial, publications derived from this study should include input from the investigator(s) and SPONSOR personnel. Such input should be reflected in publication authorship, and whenever possible, preliminary agreement regarding the strategy for order of authors' names should be established before conducting the study. Subsequent to the multicenter publication, or 24 months after completion of the study, whichever comes first, an investigator and/or his/her colleagues may publish the results for their study site independently. However, the SPONSOR does not recommend separate publication of individual study site results due to scientific concerns.

The SPONSOR must have the opportunity to review all proposed abstracts, manuscripts, or presentations regarding this study 60 days prior to submission for publication/ presentation. Any information identified by the SPONSOR as confidential must be deleted prior to submission. SPONSOR review can be expedited to meet publication guidelines.

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6. APPENDICES

6.1 LIST OF ABBREVIATIONS

AIS	Adenocarcinoma in situ
AE	Adverse experience
AN	Allocation Number
APaT	All Patients as Treated
CAP	Complete Ascertainment Program
CCS	Concomitant Cohort Study
CIN	Cervical Intraepithelial Neoplasia
CL	Center Line
cLIA	competitive Luminex immunoassay
ECC	Endocervical Curettage
FAS	Full Analysis Set
GHN	Generally HPV Naïve
GMT	Geometric Mean Titer
H & E	Hematoxylin and Eosin Stain
HPV	Human Papillomavirus
HNRT	HPV Naïve to Relevant Type
IATA	International Air Transport Association
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IgG	Immune globulin G
IRB	Institutional Review Board
LCL	Lower Control Limit
LCM	Laser Capture Microdissection
LEEP	Loop Electrosurgical Excision Procedure
LPO	Last Patient Out
LTFU	Long-Term Follow-up
MITT	Modified intent-to-treat
MRL	Merck Research Laboratories
NCC	Nordic Coordinating Center
NPP	Nordic Pathology Panel
NRSC	National Registry Study Centers
NSAE	Non-serious Adverse Experience
Pap	Papaniculaou
PCR	Polymerase Chain Reaction
PPE	Per Protocol Effectiveness
PPI	Per Protocol Immunogenicity
PRE	Pilot Registry-based Eligibility study
PIN	Subject Personal Identifier Number (similar to U.S. Social Security Number)
RCD	Reverse cumulative distribution
SAE	Serious Adverse Experience

SAP	Statistical Analysis Plan
SPR	Seropositivity Rates
SOP	Standard Operating Procedure
UCL	Upper Control Limit
VE	Vaccine Effectiveness
VLP	Virus-like Particle
WPS	Worldwide Product Safety and Epidemiology

6.2 ADAPTED POISSON SHEWHART CONTROL CHART FOR MONITORING VACCINE EFFECTIVENESS

6.2.1 Introduction

The primary objective of the V503 Nordic LTFU study is to monitor the long-term effectiveness of the 9vHPVvaccine with respect to HPV 16/18/31/33/45/52/58-related high-grade cervical disease and cancer. Control chart methods are advantageous for this objective because they can be used to evaluate vaccine effectiveness by monitoring the incidence of disease in real-time during the V503 Nordic LTFU study. Monitoring in real-time will allow for the detection of a decrease in vaccine effectiveness more quickly if effectiveness does begin to decline. Control charts have traditionally been used in industrial and manufacturing settings for process and production monitoring, but more recently these methods have been applied in epidemiology to monitor disease incidence rates in different populations over time. These methods are used in epidemiological studies for the same reason they are beneficial for monitoring vaccine effectiveness, because they allow for the prospective monitoring of disease incidence over time and can lead to quicker detection of rises in incidence rates when compared to retrospective methods.

Many control chart methods have been developed for monitoring the incidence of disease over time. Some of these methods are outlined in the review articles of Sonesson and Bock [16], Farrington and Beale [17], and Woodall [18]. Although there are many methods available in the literature, none have been found to be appropriate for the needs of the V503 Nordic LTFU study, since subjects will be monitored following their participation in a clinical trial. This scenario is unique because subjects will have completed the base study at different points during the follow-up period and they will have differing amounts of follow-up time since they received vaccine when the registry searches are conducted. An adapted Shewhart control chart was developed to monitor the long-term effectiveness of GARDASIL™. This method will also be employed for monitoring the 9vHPVvaccine to determine if and when vaccine effectiveness wanes.

The adapted Shewhart control chart method is described in Sections 6.2.2 to 6.2.5. Before presenting the adapted method, the standard Poisson Shewhart control chart methodology is described in Section 6.2.2 to provide background. The adapted method is then discussed in Section 6.2.3. In Section 6.2.4, an example of how this method will be applied in the V503 Nordic LTFU study is given. A summary of the method and comments regarding statistical properties of the adapted control chart are provided in Section 6.2.5.

6.2.2 Monitoring Using the Poisson Shewhart Control Chart

The Poisson Shewhart chart, also called the *c*-chart, is used to monitor the parameter of the Poisson distribution. The *c*-chart is a tool often used in manufacturing settings to monitor the expected number of nonconformities per unit as discussed by Montgomery [19]. In the manufacturing scenario, let X represent the number of nonconformities per unit and assume that X~Poisson(c), where c is the expected number of nonconformities per unit. To determine if the parameter c shifts, the number of nonconformities from

sampled units, *x*, can be plotted at equal time intervals on a Poisson Shewhart chart. These counts are then compared to reference lines on the chart that indicate whether the parameter *c* has shifted. The standard reference lines plotted include the center line, and upper and lower control limits. The center line represents the expected number of nonconformities and the upper and lower control limits are located *k* standard deviations above and below the center line, respectively. These values are calculated using the following formulas:

$$\text{Center Line (CL)} = c$$

$$\text{Upper Control Limit (UCL)} = c + k\sqrt{c}$$

$$\text{Lower Control Limit (LCL)} = c - k\sqrt{c}$$

To determine when *c* has shifted, each value *x* is compared to the UCL and LCL when it is plotted. If $x \geq$ UCL, then the chart signals indicating that *c* has increased. If $x \leq$ LCL, then the chart signals indicating that *c* has decreased. Traditionally, $k = 3$ so that the UCL and LCL are 3 standard deviations above and below the center line. In this case, the control limits are referred to as 3-sigma limits.

The *c*-chart described above can be modified in different applications. One modification of this chart is to use only a single control limit. This approach is taken when detecting only an increase or only a decrease in the expected number of nonconformities is of interest. In these cases only the UCL or LCL is needed and the control chart is referred to as a one-sided chart. A second modification to the *c*-chart is to use one or more supplementary signaling rules often called runs rules. These are additional criteria that would produce a signal in addition to the signaling rule outlined above using the UCL and LCL. Some examples of some common runs rules applied when 3-sigma limits are used are:

- Two out of three consecutive points ≥ a 2-sigma limit above the CL but below the UCL or two out of three consecutive points ≤ a 2-sigma limit below the CL but above the LCL.

- Four out of five consecutive points ≥ a 1-sigma limit above the CL but below the UCL or four out of five consecutive points ≤ a 1-sigma limit below the CL but above the LCL.

- Eight consecutive points above the CL or below CL.

The *c*-chart can be used similarly to monitor disease incidence counts or rates, which would indicate if vaccine effectiveness has shifted, assuming the counts follow a Poisson distribution. This would be done by plotting estimates of disease incidence counts or disease incidence rates over time for intervals with equal amounts of subject follow-up time. After each point is plotted, it would be compared to an UCL and LCL to determine

if a signal is produced. To determine if there is a decrease in vaccine effectiveness, only an UCL would be needed to detect increases in the disease incidence rate.

6.2.3 Adapted Poisson Shewhart Control Chart

Although the *c*-chart described in Section 6.2.2 can be used to monitor vaccine effectiveness, it can only be used to monitor vaccine effectiveness in subjects after the completion of a clinical trial if the following conditions are met:

- All subjects monitored are vaccinated at the same time so that all subjects will have had the same amount of follow-up time since vaccination when the disease incidence count estimates for each control chart interval are determined.

- All subjects are assessed for disease at the end of each time interval on the control chart so that there is equal subject follow-up time contributing to each estimate.

The first criterion must be met in order to monitor vaccine effectiveness from the time the vaccine is administered, which is needed to determine if and when vaccine effectiveness wanes. If subjects are vaccinated on different dates, then estimates plotted on the control chart at the end of each time interval will be calculated from subjects at different points following vaccination. In this case, the estimates and potential signals on the control chart would be difficult to interpret. The second criterion must be met so that the control limits and CL are correct as they are based on the assumption that there are equal amounts of follow-up time in each interval.

In the V503 Nordic LTFU study, neither of the two criteria above can be met. Since subjects were enrolled into the base study at different times, the time since vaccine administration will not be the same for each subject at the time of each registry search. In the traditional application of the *c*-chart, an estimate would be plotted on the control chart corresponding to the date in time when data are collected. In the V503 Nordic LTFU study analysis, it is important that the estimates be calculated and plotted based on time since vaccination to determine if and when vaccine effectiveness wanes.

In addition to differing enrollment dates with respect to the base study, subjects also completed the trial at different times since the end of their vaccination series and will be screened somewhat arbitrarily over the 10-year monitoring period based on when the subjects choose to schedule visits. Since the disease incidence estimates will be calculated based on time since vaccination, the available data following each registry search will be right- and left-censored. As a result, the amount of follow-up time corresponding to the time intervals on the control chart will differ for a single analysis, and the amount of data available for each time interval will change for each analysis as more registry searches are performed. In standard control chart monitoring scenarios, all information for an estimate plotted on the chart is obtained before the point is plotted. In the V503 Nordic LTFU study, data are collected in real-time, but are plotted in time relative to vaccination. Therefore, each time the registries are searched, more data may become available for a particular time interval.

To manage the unique challenges of this application, two adjustments are needed to the standard application of the *c*-chart so that it can be used for monitoring vaccine effectiveness in the V503 Nordic LTFU study. First, the CL and control limits must be adjusted for each time interval in each analysis to account for differing amounts of follow-up time, which changes the expected disease incidence count and the variance in each interval. In addition, the disease incidence estimates in each time interval will need to be updated each time a new analysis is conducted as more data become available. To incorporate these changes to the *c*-chart, let *t* represent the total number of time intervals on the control chart and let *s* represent the total number of analyses conducted, i.e. the number of times data are collected, then the CL and control limits can be calculated using the following formulas:

$$CL_{ij} = c_{ij}$$

$$UCL_{ij} = c_{ij} + k\sqrt{c_{ij}}$$

$$LCL_{ij} = c_{ij} - k\sqrt{c_{ij}}$$

where $i = 1, 2, \ldots, t$ and $j = 1, 2, \ldots, s$. In this case, x_{ij} would be the disease incidence estimate plotted for the *i*th interval in the *j*th analysis. The control chart would produce a signal if $x_{ij} \geq UCL_{ij}$ or $x_{ij} \leq LCL_{ij}$. As with the standard *c*-chart, this control chart can be one-sided and/or incorporate runs rules.

An additional consideration in this application is whether or not to truncate the control chart on either the left or right side, or both sides. There will be a limited amount of follow-up time contributing to the estimates for the left- and right-most time intervals. If limited data are available for these intervals, the disease incidence estimates may be difficult to interpret and may lead to incorrect conclusions regarding waning effectiveness. This is discussed in more detail in Section 6.2.4.

6.2.4 Application of the Adapted Poisson Shewhart Control Chart in the Context of the V503 Nordic LTFU Study

To show how the adapted *c*-chart will be used in the V503 Nordic LTFU study, an example is provided here. In this study, subjects from Denmark, Norway, and Sweden will be monitored over the 10-year period following the base clinical study. Subjects are expected to schedule screening visits approximately 3 years apart. Detailed information has been provided by the Nordic Cancer Registries to accurately simulate subject visit schedules and the outcomes for each visit over the 10-year follow-up period. In addition, the Nordic Cancer Registries have provided data to determine the baseline rate of high-grade cervical lesions related to HPV types 16/18/31/33/45/52/58. The baseline rate is assumed to be 0.438/1000 person-years in recipients, which corresponds to 90% effectiveness. A baseline rate based on 90% effectiveness is being used to detect any decrease in vaccine effectiveness below 90%. The visit outcomes in the simulated data used for the example are based on an underlying vaccine effectiveness model that starts at 90% at the beginning of the monitoring period and decreases linearly to 50% the end of

the 10-year monitoring period. This results in an average vaccine effectiveness of 70% over the 10-year follow-up period. Data were simulated for subjects in the base study.

There will be 5 control chart analyses over the 10-year period, one every two years. Analyses are planned at two-year intervals based on the expected visit schedule. To ensure enough follow-up time is accrued for the disease incidence estimates in each time interval, the width of the time intervals on the control chart will also be two years. Since the goal of the V503 Nordic LTFU study is to determine waning vaccine effectiveness, a one-sided control chart will be used. In the example shown here, the adapted *c*-chart will signal if one point crosses the 2.75-sigma UCL. A runs rule will also be applied, where a signal will also occur if two out of three consecutive points cross the 1.83-sigma UCL.

For each subject contributing to the control chart analyses, the V503 Nordic LTFU study begins when the subject exits the Protocol V503-001 base study. The base study had scheduled visits through Month 42, 48, or 54 depending on when the subject enrolled, which is approximately 4 years following a subject's first vaccination. Although some subjects exited the study before 4 years had elapsed, there will be limited follow-up information prior to Year 4. Therefore, it is reasonable to begin monitoring vaccine effectiveness at Year 4 in this application. In this case, the control chart will be truncated on the left and any incidence count estimates prior to Year 4 should not be plotted. For illustrative purposes, these estimates will be shown in this example, but the intervals will be shaded to indicate there is limited follow-up time accrued.

In the V503 Nordic LTFU study control chart analyses, there is potential for false-positive and false-negative signals. A false-positive signal occurs if a signal is seen in an interval for one analysis and then the signal no longer exists in a subsequent analysis when more follow-up time is accrued. A false-negative signal occurs if a signal is not present in an interval in an analysis, but then a signal appears in the interval in a subsequent analysis. False-positive and false-negative signals can occur randomly, but are also influenced by this particular application. Although we assume a Poisson distribution for our disease incidence counts, data are simulated based on the expected visit schedule and probabilities of different outcomes at these visits, as provided by the Nordic Cancer Registries. Therefore, the simulated data are only approximately Poisson distributed, which impacts the probability of the occurrence of a false-positive or false-negative signal. False-positive and false-negative signals tend to occur when a point is estimated and plotted for an interval with limited follow-up time accrued. Due to the right-censored data, this will occur in the right-most intervals of the control chart in the analyses for this study. Therefore, in this application, points should not be plotted when there is limited follow-up time in an interval. Simulation results have shown that for the V503 Nordic LTFU study, at least 60% of the total expected person-years of follow-up time in each interval should be accrued before plotting an estimate for the interval, with one exception. If the percentage of follow-up time accrued is less than 60%, but the number of breakthrough cases is large enough where it is clear a signal would be detected once 60% or more of the follow-up time is accrued, then this point should be plotted on the control chart. If points are plotted as described, the probability of a false-positive or false-negative signal is negligible based on the simulation results. Although it is

recommended to leave estimates off of the control chart when limited follow-up time is accrued, in this example, these points will be plotted in order to demonstrate the method. When this occurs, the intervals will be shaded to indicate that the results are calculated from limited follow-up information.

Figure 6-1 shows the first control chart analysis based on the simulated data. The first analysis period occurs after only 2 years of follow-up data are collected and with visits occurring on the 3-year schedule. Therefore, the control chart is only based on a limited amount of data at the first analysis. In this example, all intervals are shaded, indicating that there is not enough person-time accrued to interpret the estimates on the chart. Even though there is limited follow-up time accrued, it is evident that there is differing follow-up time in each interval, based on the changing center line and control limit values for each interval.

Figure 6-2 shows the second simulated analysis. Here there are no signals. Note that there are two cases in the interval from 6 to 8 years. This interval is shaded because less than 60% of the follow-up time in this interval has been accrued. This interval could potentially have a false-negative signal and is only plotted here to illustrate the potential for this type of error if cases are assessed with limited follow-up information.

Figure 6-3 shows the third analysis, where there are still no signals. This is reasonable, given the underlying vaccine effectiveness model and that many subjects have only completed visits early in the follow-up period. The number of cases exceeds the 2.75-sigma limit between Year 8 and 10, as well as between Year 10 and 12, but these intervals are shaded due to limited follow-up time. Similar to the second analysis in Figure 6-2, these could potentially be false-positive signals and are only plotted here to illustrate the potential for this type of error if cases are assessed with limited follow-up information.

Figure 6-4 shows the fourth analysis. There are still no signals here, but there is one point between the 1.83- and 2.75-sigma limits, which could contribute to a future signal. This analysis is very similar to the third analysis in regard to the potential false-positive signals.

Figure 6-5 shows the fifth analysis. As with the previous analyses, there are still no signals in intervals with at least 60% follow-up time.

Figure 6-6 shows the sixth and final analysis. Here there are two points above the 1.83-sigma limit between Year 8 and 10 and Year 12 and 14. In addition, there are now 7 cases in the interval from 14 to 16 years, which produces another signal over the 2.75-sigma limit. Either of these signals would require the initiation of a discussion regarding waning effectiveness with regulatory agencies. Based on the underlying vaccine effectiveness model, there has been a shift in vaccine effectiveness at this point and this example shows the ability of the control chart to detect this shift.

Figure 6-1

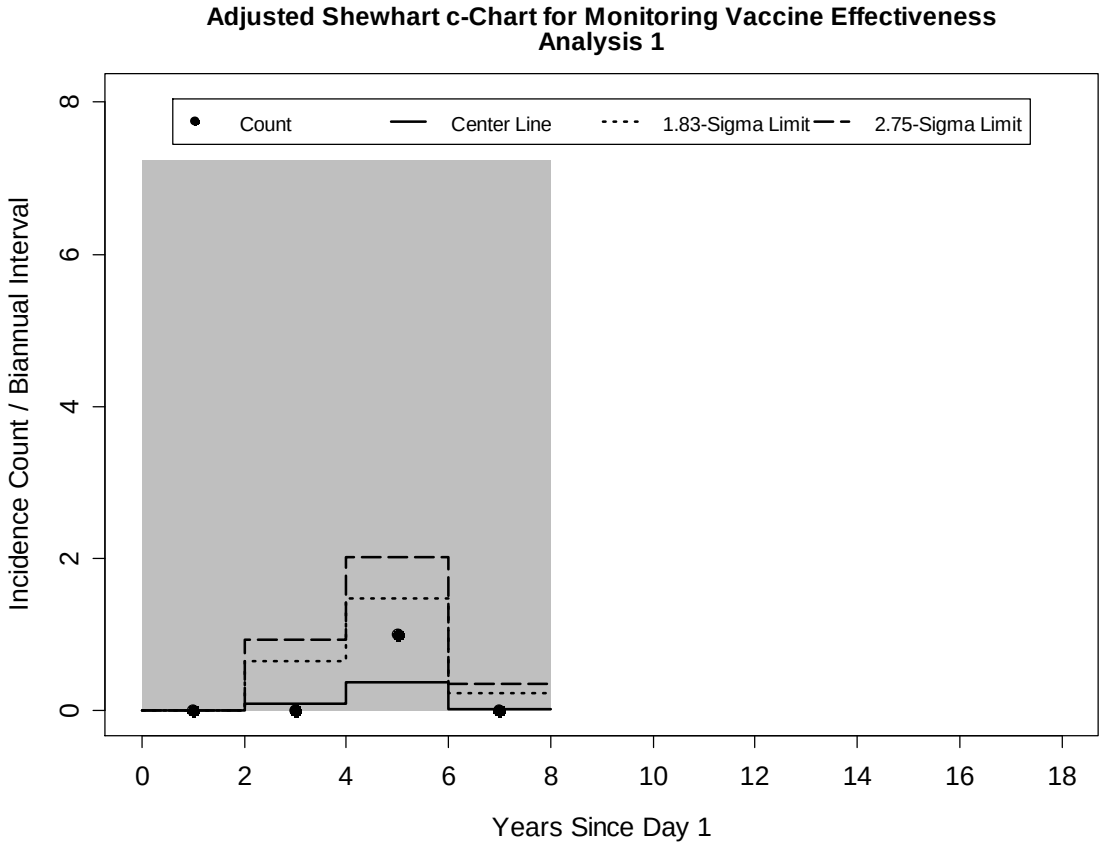

Adjusted Shewhart c-Chart for Monitoring Vaccine Effectiveness
Analysis 1
Count
Center Line
1.83-Sigma Limit
2.75-Sigma Limit
Incidence Count / Biannual Interval
0
2
4
6
8
0
2
4
6
8
10
12
14
16
18
Years Since Day 1

Figure 6-2

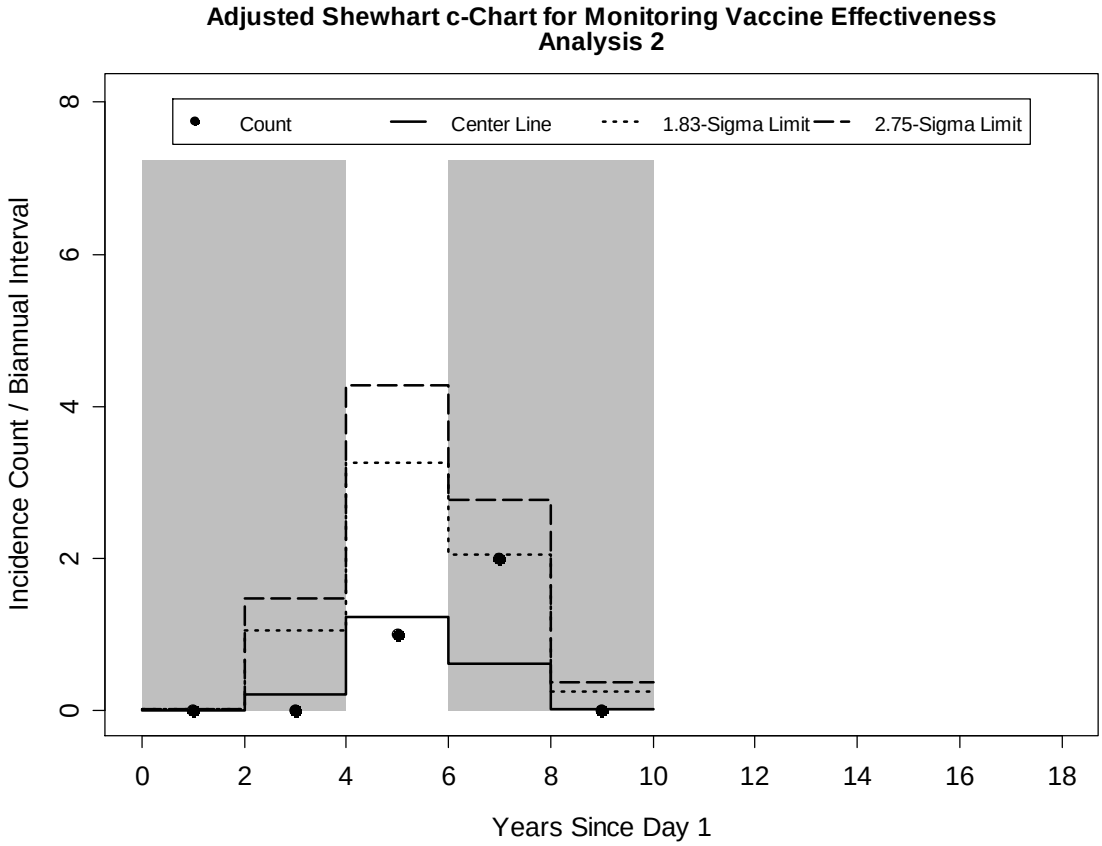

Adjusted Shewhart c-Chart for Monitoring Vaccine Effectiveness
Analysis 2
Count
Center Line
1.83-Sigma Limit
2.75-Sigma Limit
Incidence Count / Biannual Interval
0
2
4
6
8
0
2
4
6
8
10
12
14
16
18
Years Since Day 1

Figure 6-3

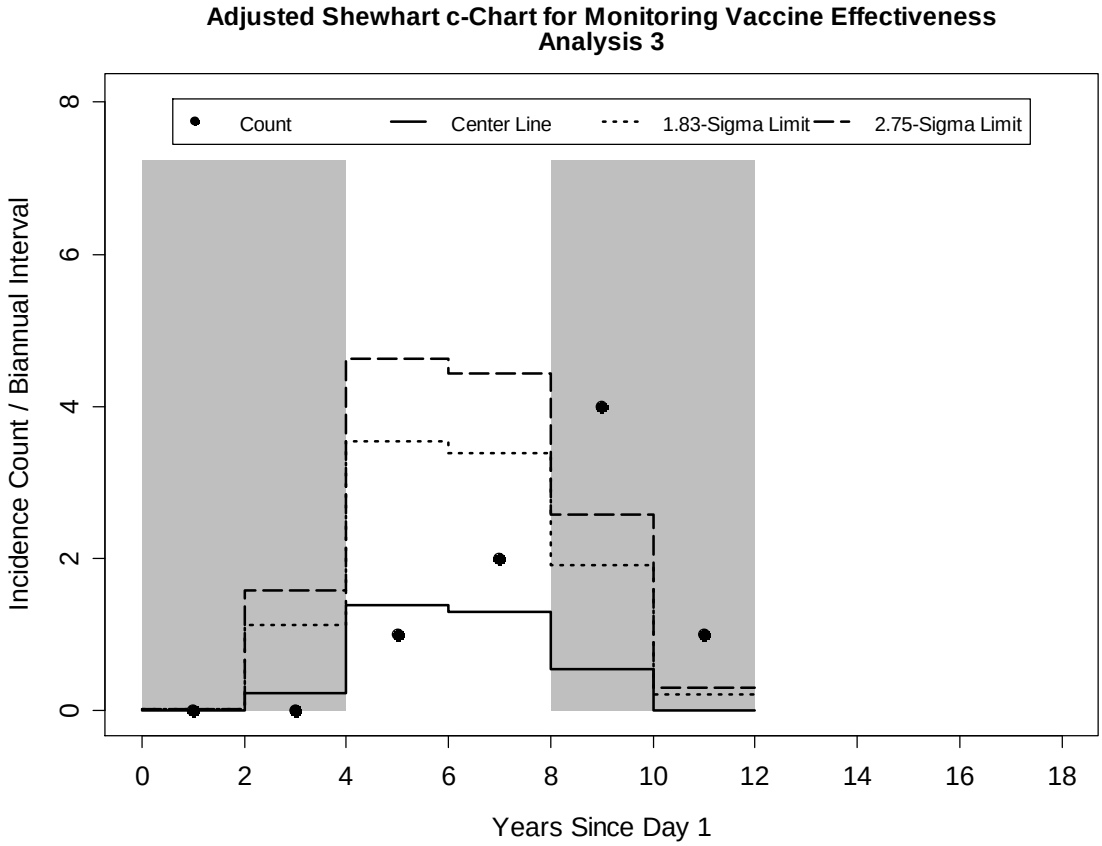

Adjusted Shewhart c-Chart for Monitoring Vaccine Effectiveness
Analysis 3
Count
Center Line
1.83-Sigma Limit
2.75-Sigma Limit
Incidence Count / Biannual Interval
0
2
4
6
8
0
2
4
6
8
10
12
14
16
18
Years Since Day 1

Figure 6-4

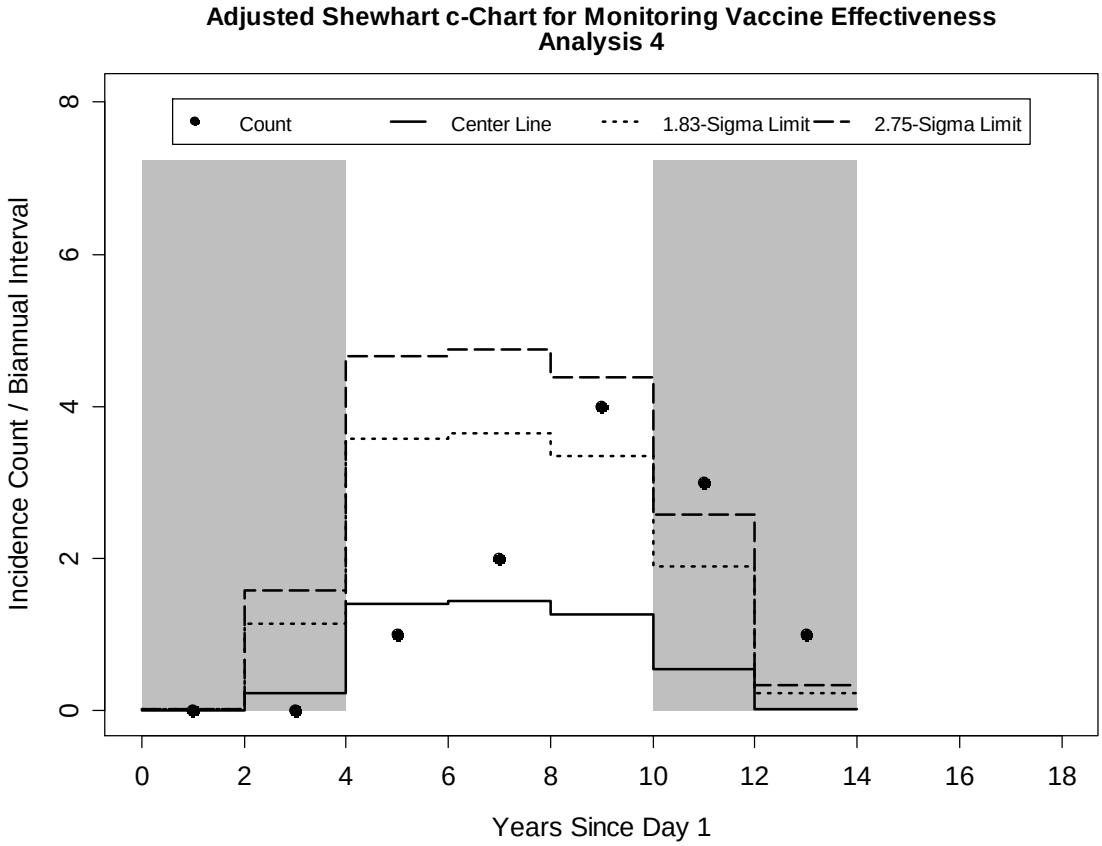

Adjusted Shewhart c-Chart for Monitoring Vaccine Effectiveness
Analysis 4
Count
Center Line
1.83-Sigma Limit
2.75-Sigma Limit
Incidence Count / Biannual Interval
0
2
4
6
8
0
2
4
6
8
10
12
14
16
18
Years Since Day 1

Figure 6-5

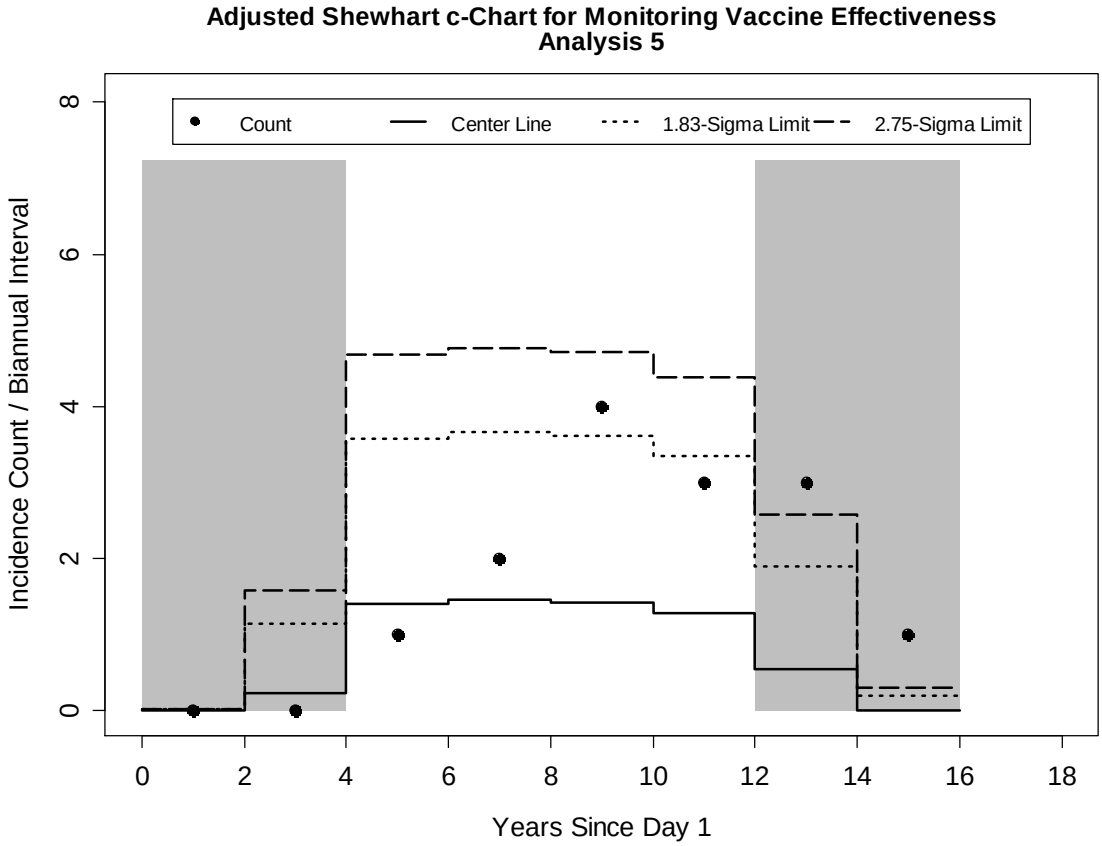
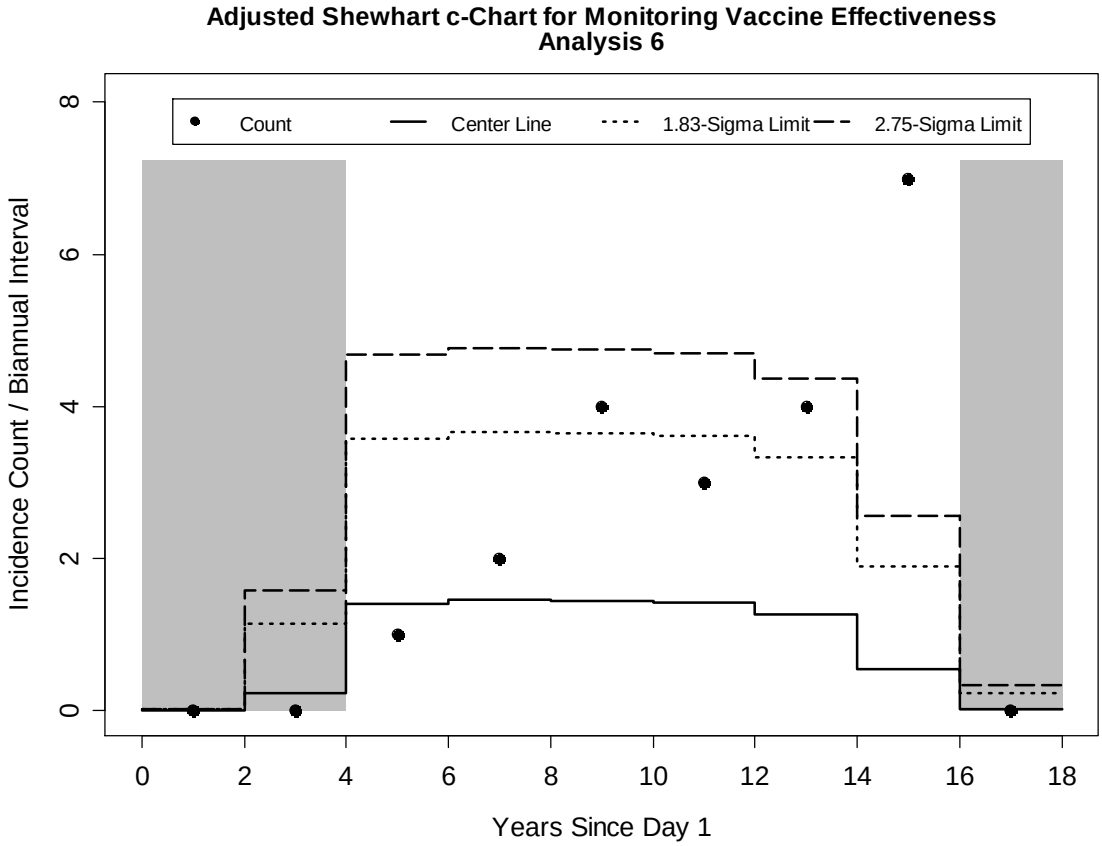

Adjusted Shewhart c-Chart for Monitoring Vaccine Effectiveness
Analysis 5
Count
Center Line
1.83-Sigma Limit
2.75-Sigma Limit
Incidence Count / Biannual Interval
0
2
4
6
8
0
2
4
6
8
10
12
14
16
18
Years Since Day 1

Figure 6-6


Adjusted Shewhart c-Chart for Monitoring Vaccine Effectiveness
Analysis 6
Count
Center Line
1.83-Sigma Limit
2.75-Sigma Limit
Incidence Count / Biannual Interval
0
2
4
6
8
0
2
4
6
8
10
12
14
16
18
Years Since Day 1

6.2.5 Summary and Discussion of Statistical Properties

The details of the methodology that will be used for the analyses in the V503 Nordic LTFU study have been presented in Sections 6.2.2 – 6.2.4. This method is an adaptation of the standard Shewhart *c*-chart, where the *c*-chart has been adapted to monitor the expected disease incidence count when there is unequal follow-up time corresponding to each time interval on the control chart and when estimates are plotted on a different time scale than the time of data collection. The adjustments needed to handle the differing time scales for the control chart and data collection are issues that have not been widely addressed in control chart applications. It is, however, becoming more common as the use of control charts broadens in healthcare applications.

The performance of a control chart is important to consider in each application. The performance indicates the statistical properties of the chart, including how often it is expected to signal when there is a shift in the monitored parameter and when there is no shift. The example provided in Section 6.2.4 uses a 2.75-sigma control limit and a runs rule based on a 1.83-sigma control limit. The performance of the adapted *c*-chart is based on the control limits and signaling rule(s) used. Traditionally, control charts are used to analyze incoming data over time until a signal is produced. Once the chart signals, the cause of the parameter shift is investigated. In these scenarios, the typical performance measures are the in-control and out-of-control average run lengths of the chart. These are the average number of estimates plotted until a signal is produced when there is no shift in the monitored parameter and when there is a shift in the monitored parameter, respectively. In the V503 Nordic LTFU study, estimates will be plotted a prespecified number of times. In this case, describing the performance of the control chart based on the probability of a signal over the prespecified number of analyses is most appropriate. The probability of a signal when the monitored parameter has not shifted can be considered the alpha-level of the control chart in this case. The probability of a signal when there is a shift in the monitored parameter would be considered the power of the control chart.

Due to the adjustments made to this control chart for application in the V503 Nordic LTFU study, properties of the control chart must be determined through simulation. If one wishes to control the type I error rate of the control chart, simulations should be done to search for settings of the control limits and signaling rules to achieve the desired alpha-level. After control limits and signaling rules are determined that provide an acceptable alpha-level, the power at these settings can be assessed by simulation.

Simulations can also be used to determine if and when the left- and right-most intervals on the control chart should be truncated due to a limited amount of subject follow-up time in these intervals. Rules for truncation can be established to control the false-positive and false-negative rates of the control chart.

7. ATTACHMENTS

Merck Code of Conduct for Clinical Trials

Merck*
Code of Conduct for Clinical Trials

I. Introduction

A. Purpose

Merck, through its subsidiaries, conducts clinical trials worldwide to evaluate the safety and effectiveness of our products. As such, we are committed to designing, implementing, conducting, analyzing and reporting these trials in compliance with the highest ethical and scientific standards. Protection of subject safety is the overriding concern in the design of clinical trials. In all cases, Merck clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

B. Scope

Such standards shall be endorsed for all clinical interventional investigations sponsored by Merck irrespective of the party (parties) employed for their execution (e.g., contract research organizations, collaborative research efforts). This Code is not intended to apply to trials which are observational in nature, or which are retrospective. Further, this Code does not apply to investigator-initiated trials which are not under the control of Merck.

II. Scientific Issues

A. Trial Conduct

1. Trial Design

Except for pilot or estimation trials, clinical trial protocols will be hypothesis-driven to assess safety, efficacy and/or pharmacokinetic or pharmacodynamic indices of Merck or comparator products. Alternatively, Merck may conduct outcomes research trials, trials to assess or validate various endpoint measures, or trials to determine subject preferences, etc.

The design (i.e., subject population, duration, statistical power) must be adequate to address the specific purpose of the trial. Research subjects must meet protocol entry criteria to be enrolled in the trial.

2. Site Selection

Merck selects investigative sites based on medical expertise, access to appropriate subjects, adequacy of facilities and staff, previous performance in Merck trials, as well as budgetary considerations. Prior to trial initiation, sites are evaluated by Merck personnel to assess the ability to successfully conduct the trial.

3. Site Monitoring/Scientific Integrity

Trial sites are monitored to assess compliance with the trial protocol and general principles of Good Clinical Practice. Merck reviews clinical data for accuracy, completeness and consistency. Data are verified versus source documentation according to standard operating procedures. Per Merck policies and procedures, if fraud, misconduct or serious GCP-non-Compliance are suspected, the issues are promptly investigated. When necessary, the clinical site will be closed, the responsible regulatory authorities and ethics review committees notified and data disclosed accordingly.

B. Publication and Authorship

To the extent scientifically appropriate, Merck seeks to publish the results of trials it conducts. Some early phase or pilot trials are intended to be hypothesis-generating rather than hypothesis testing. In such cases, publication of results may not be appropriate since the trial may be underpowered and the analyses complicated by statistical issues of multiplicity.

Merck's policy on authorship is consistent with the requirements outlined in the ICH-Good Clinical Practice guidelines. In summary, authorship should reflect significant contribution to the design and conduct of the trial, performance or interpretation of the analysis, and/or writing of the manuscript. All named authors must be able to defend the trial results and conclusions. Merck funding of a trial will be acknowledged in publications.

III. Subject Protection

A. IRB/ERC review

All clinical trials will be reviewed and approved by an independent IRB/ERC before being initiated at each site. Significant changes or revisions to the protocol will be approved by the IRB/ERC prior to implementation, except that changes required urgently to protect subject safety and well-being may be enacted in anticipation of IRB/ERC approval. For each site, the IRB/ERC and Merck will approve the subject informed consent form.

B. Safety

The guiding principle in decision-making in clinical trials is that subject welfare is of primary importance. Potential subjects will be informed of the risks and benefits of, as well as alternatives to, trial participation. At a minimum, trial designs will take into account the local standard of care. Subjects are never denied access to appropriate medical care based on participation in a Merck clinical trial.

All participation in Merck clinical trials is voluntary. Subjects are enrolled only after providing informed consent for participation. Subjects may withdraw from a Merck trial at any time, without any influence on their access to, or receipt of, medical care that may otherwise be available to them.

C. Confidentiality

Merck is committed to safeguarding subject confidentiality, to the greatest extent possible. Unless required by law, only the investigator, sponsor (or representative) and/or regulatory authorities will have access to confidential medical records that might identify the research subject by name.

D. Genomic Research

Genomic Research will only be conducted in accordance with informed consent and/or as specifically authorized by an Ethics Committee.

IV. Financial Considerations

A. Payments to Investigators

Clinical trials are time- and labor-intensive. It is Merck's policy to compensate investigators (or the sponsoring institution) in a fair manner for the work performed in support of Merck trials. Merck does not pay incentives to enroll subjects in its trials. However, when enrollment is particularly challenging, additional payments may be made to compensate for the time spent in extra recruiting efforts.

Merck does not pay for subject referrals. However, Merck may compensate referring physicians for time spent on chart review to identify potentially eligible subjects.

B. Clinical Research Funding

Informed consent forms will disclose that the trial is sponsored by Merck, and that the investigator or sponsoring institution is being paid or provided a grant for performing the trial. However, the local IRB/ERC may wish to alter the wording of the disclosure statement to be consistent with financial practices at that institution. As noted above, publications resulting from Merck trials will indicate Merck as a source of funding.

C. Funding for Travel and Other Requests

Funding of travel by investigators and support staff (e.g., to scientific meetings, investigator meetings, etc.) will be consistent with local guidelines and practices including, in the U.S., those established by the American Medical Association (AMA).

V. Investigator Commitment

Investigators will be expected to review Merck's Code of Conduct as an appendix to the trial protocol, and in signing the protocol, agree to support these ethical and scientific standards.

* In this document, "Merck" refers to Merck Sharp & Dohme Corp. and Schering Corporation, each of which is a subsidiary of Merck & Co., Inc. Merck is known as MSD outside of the United States and Canada. As warranted by context, Merck also includes affiliates and subsidiaries of Merck & Co., Inc."

8. SIGNATURES

8.1 SPONSOR'S REPRESENTATIVE

TYPED NAME	SIGNATURE	DATE

8.2 INVESTIGATOR

I agree to conduct this clinical study in accordance with the design outlined in this protocol and to abide by all provisions of this protocol (including other manuals and documents referenced from this protocol); deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment. I agree to conduct the study in accordance with generally accepted standards of Good Clinical Practice. I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences as defined in the SAFETY MEASUREMENTS section of this protocol. I also agree to handle all clinical supplies provided by the SPONSOR and collect and handle all clinical specimens in accordance with the protocol. I understand that information that identifies me will be used and disclosed as described in the protocol, and that such information may be transferred to countries that do not have laws protecting such information. Since the information in this protocol and the referenced Investigator's brochure is confidential, I understand that its disclosure to any third parties, other than those involved in approval, supervision, or conduct of the study is prohibited. I will ensure that the necessary precautions are taken to protect such information from loss, inadvertent disclosure, or access by third parties.

TYPED NAME	SIGNATURE	DATE