STUDY INFORMATION

Title:	Post-marketing study to assess the risk of intussusception after immunization with GlaxoSmithKline (GSK) Biologicals' oral live-attenuated human rotavirus vaccine in infants less than 1 year old in Latin America.
Protocol version identifier:	212329 EPI-ROTA-070 VS LAT PA v1.1
Date of last version of the protocol:	Original protocol: 18 October 2021 Protocol Amendment 1.0: 18 July 2024 Protocol Amendment 1.1: 26 February 2025
EU PAS Register No:	Not applicable
Active substance:	Human rotavirus, live-attenuated (Anatomical Therapeutic Chemical (ATC) code: J07BH01)
Medicinal product(s):	GlaxoSmithKline Biologicals SA (GSK) human rotavirus vaccine, live, oral (HRV) (*Rotarix*)
Product reference:	BLA 125265
Procedure number:	Not applicable
Marketing Authorization Holder(s) (MAH):	GlaxoSmithKline Biologicals SA
Joint PASS:	No
Research question and objectives:	To evaluate post-authorization, the risk of definite intussusception (IS) following immunization with *Rotarix* porcine circovirus (PCV)-free liquid vaccine in infants less than 1 year old in Latin America (LATAM). Primary Objective: To assess the risk of definite IS within 7 days following immunization with the first dose of *Rotarix* PCV-free liquid vaccine Secondary Objectives:

	To assess the risk of definite IS within 7 days following immunization with the second dose of *Rotarix* PCV-free liquid vaccine To assess the risk of definite IS during 3 risk windows following immunization with the first dose of *Rotarix* PCV-free liquid vaccine accounting for time-varying confounders
Countries of study:	A total of 5 to 8 countries across LATAM
Author:	PPD Vaccine Epidemiology; Viral Non-Respiratory GlaxoSmithKline Biologicals SA Rue de l'Institut, 89 B-1330 Rixensart Belgium

MARKETING AUTHORISATION HOLDER

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Based on GSK Biologicals' protocol template for post-authorization safety studies v17.1

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2. LIST OF ABBREVIATIONS

AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
CBER	Center for Biologics Evaluation and Research
CRF/eCRF	Case Report Form/electronic Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
EDC	Electronic Data Capture
EMA	European Medicines Agency
EMRs	Electronic Medical Records
EoS	End of Study
ER	Emergency Room
FDA	Food and Drug Administration
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GPP	Good Pharmacoepidemiology Practices
GSK	GlaxoSmithKline Biologicals SA
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRV	Human Rotavirus Vaccine
ICE	Intercurrent Events
ICF	Informed Consent Form
ICH	International Council on Harmonization
ICMJE	International Committee of Medical Journal Editors
IEC	Independent Ethics Committee

IPV	Inactivated Poliovirus Vaccine
IRB	Institutional Review Board
IS	Intussusception
ISPE	International Society for Pharmacoepidemiology
LAR	Legally Acceptable Representative
LATAM	Latin America
MAH	Marketing Authorization Holder
NIP	National immunization programs
OPV	Oral polio vaccine
PAHO	Pan American Health Organization
PASS	Post-Authorization Safety Study
PBRER	Periodic Benefit Risk Evaluation Report
PCD	Primary Completion Date
PCV	Porcine Circovirus
RRV-TV	Rhesus-Human Reassortant Rotavirus Vaccine
RV	Rotavirus
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SCRI	Self-Controlled Risk Interval
US	United States
WHO	World Health Organization

3. RESPONSIBLE PARTIES

The list of main responsible parties, including principal investigator and coordinating investigator for each country in which the study is to be performed and their corresponding contact details will be kept in a standalone document.

4. ABSTRACT

Title	Post-marketing study to assess the risk of intussusception after immunization with GlaxoSmithKline Biologicals (GSK) oral live-attenuated human rotavirus vaccine in infants less than 1 year old in Latin America.
Version and date of the protocol	Original protocol: 18 October 2021 Protocol Amendment v1.0: 18 July 2024 Protocol Amendment v1.1: 26 February 2025
Main author	PPD Vaccine Epidemiology; Viral Non-Respiratory GlaxoSmithKline Biologicals SA Rue de l'Institut, 89 B-1330 Rixensart Belgium
Rationale and background	Rotavirus (RV) is the main cause of acute gastro-enteritis and severe diarrhea among young children (<5 years old) in both developing and developed countries; however, there are marked differences in the burden of RV disease, with highest mortality seen in low- and middle-income countries. RV vaccines have been shown to have significant positive impact on diarrheal morbidity and mortality in diverse geographies. Since the introduction of the RV vaccine into national immunization programs (NIP), the number of deaths from RV in children (<5 years old) has declined by nearly 60% in approximately 10 years, with an estimated 122 000 to 215 000 deaths annually observed during the period 2013 to 2017 compared to 528 000 deaths in 2000 [WHO, 2021; ROTA council, 2020]. Although RV vaccines are generally well tolerated, they have been associated with a transient increase in the risk of intussusception (IS) [WHO, 2021]. As part of the requirements for licensure of *Rotarix* porcine circovirus (PCV)-free[1] liquid vaccine in United States (US), the Center for Biologics Evaluation and Research (CBER) requested GlaxoSmithKline Biologicals SA (GSK) to conduct a post-marketing study to assess the risk of IS post-vaccination.
Research question and objectives	**Research Questions**: To evaluate post-authorization, the risk of definite IS following immunization with *Rotarix* PCV-free liquid vaccine in infants less than 1 year old in Latin America (LATAM). **Primary Objective**: To assess the risk of definite IS within 7 days following immunization with the first dose of *Rotarix* PCV-free liquid vaccine **Secondary Objectives:** • To assess the risk of definite IS within 7 days following immunization with the second dose of *Rotarix* PCV-free liquid vaccine • To assess the risk of definite IS during 3 risk windows following immunization with the first dose of *Rotarix* PCV-free liquid vaccine accounting for time-varying confounders.
Study design	A hospital-based, observational, multicenter, prospective matched case-control study.
Population	**Cases**: IS cases will include all infants hospitalized or visiting the hospital emergency room (ER) for definite IS at the participating hospitals during the study period and aged between 6 weeks and 1 year of age at the time of diagnosis of IS. Cases will be identified by periodic reviews of pediatric ward admission and ER logbook(s). **Controls**: For each case, 6 controls hospitalized or visiting the hospital outpatient clinic or ER with no gastro-intestinal symptoms (including gastro-enteritis and IS) in the same participating hospital, on the same day as the case (±7 days), will be included after matching by age (±2 weeks) at index date[2]. The

	investigator should make every effort to select suitable age-matched controls as soon as a case is identified.
Endpoints	**Primary endpoint**: Number of cases (and of controls, separately) exposed to a first dose of RV vaccination within 7 days prior to index date[2]. **Secondary endpoints**: • Number of cases (and of controls, separately) exposed to a second dose of RV vaccination within 7 days prior to index date • Occurrence of IS during one of the four intervals: Day 1 to 7, Day 8 to 14, Day 15 to 21, and Day 22 to 35 after first dose of RV vaccine among vaccinated subjects.
Data sources	Medical records, parent(s)/legally acceptable representative (LAR[s]) interviews, vaccination cards and immunization registries.
Study size	Approximately 229 cases and 1374 controls (considering a case-control ratio 1:6).
Data analysis	Statistical methods will be fully described in detail in a statistical analysis plan (SAP).
Anticipated Milestones	Start of Data Collection: Study start date will depend on the launch of *Rotarix* PCV-free liquid vaccine in the participating countries. End of Data Collection: Study duration is expected to be 2 years from start of data collection. End of Study: End of Study (EoS) is defined as completion date of statistical analysis. Final report of study result: Clinical Study Report (CSR) will be submitted to CBER within 1 year of the end of the study.

1 PCV-free is defined as 'No detection of PCV-1 and PCV-2 according to the limit of detection of the tests used'.
2 The index date will be the onset date of the definite IS episode for cases and the hospital admission date or outpatient or/ ER visit date for controls.

5. AMENDMENTS AND UPDATES

Amendment or Update No	Date	Section of Study Protocol	Amendment or Update	Reason
1	18 Jul 2024	4. Abstract; 8.2 Secondary objectives; 9.1.4 Study endpoints; 9.5.2 Sample size for self-control interval analysis; 9.7.2.2 Analysis dataset for the self-controlled risk interval analysis; 9.7.3.3 Secondary endpoints analyses; 9.9 Limitations of the research methods	Self-controlled risk interval (SCRI) analysis that was originally planned as a sensitivity analysis was added as a secondary endpoint to the study. A secondary objective was added corresponding to the SCRI analysis	FDA recommendation
		4. Abstract; 9.7.2.2 Analysis dataset for the self-controlled risk interval analysis; 9.73.3. Secondary endpoints analyses	Added 2 additional risk windows in the SCRI analysis: Days 8–14, and Days 15–21; and shortened the control window to Day 22 to Day 35	FDA recommendation
		8.3 Estimand framework	A table with estimand framework was added	Estimand framework was added to summarize: the treatment or exposure and population to be investigated, the endpoint and how it will be estimated, and strategy for intercurrent events.
		9.1.7 Summary of study feasibility	Section was updated to reflect the findings from a feasibility study performed in 2024	A study to assess feasibility was performed resulting in new findings.
		Throughout the document	Number of participating countries change to 5-8 based on findings from the feasibility study	Change based on findings from the feasibility study.
		9.5 Study size	Recalculated sample size for the primary analysis: 229 for cases and 1374 for controls. Recalculated sample size for the SCRI analysis as well	The number of countries was updated based on the feasibility study, and the percent vaccination coverage was updated. As a result, the sample size was re-calculated. For the SCRI analysis, 2 additional risk windows are added, and the period for the control window was shortened. As a result, the sample size for this analysis was also re-calculated.
		9.7 Data analysis	Added description in the analysis that patients with OPV co-administration will be excluded from the main analysis	Add clarification per FDA recommendation

Amendment or Update No	Date	Section of Study Protocol	Amendment or Update	Reason
1.1	26 Feb 2025	9.2.4 Subject withdrawal	Updated the description of Subject withdrawal and those who are discontinued	Add clarification of withdrawal of consent and discontinuation of patients whose vaccination status cannot be verified through a source
		9.2.5.1 Cases	Added ascertainment of definite IS with an adjudication process	Clarify how definite case will be confirmed to ensure accuracy and consistency of the evaluation of the definite IS diagnosis
		9.3.1 Variable collected at enrollment, 9.3.2 Variables collected during contact follow-up, and Table 4	Updated that data collected under 'Diagnostic procedures and final diagnosis' includes any records related to definite IS diagnosis, which will be collected as defined in the clinical event committee charter	Clarified that any records related to definite IS diagnosis in the medical record will be collected at any timepoint in the study
		9.1.1 Overall design, 9.2.6 Study assessments and data collection, 9.3.1 Variables collected at enrollment, Table 4, and 9.7.3.2 Primary endpoints analyses	Added 'race' next to the ethnicity variable (must read "race and / or ethnicity" instead of "ethnicity" alone) in the demographic data collected for the study. Consistently, ethnicity variable was adapted to "race and / or ethnicity" as potential parameter in the logistic regression model for the primary endpoints.	Race and ethnicity are 2 concepts closely related. Clarified that race and ethnicity will be collected if available and per local regulation as might be more adapted in the countries where the study will be conducted.
		9.7.3.2 Primary endpoint analyses	Added "(but may not limited to)" regarding the potential confounders and effect measure modifiers that will be fitted in the logistic regression model.	Clarified that gender, race or ethnicity (if available) and feeding status may be included in the model, however, this may not be the exhausted list and will be further described in the SAP.

FDA: Food and Drug Administration; OPV: oral polio vaccine

6. MILESTONES

Anticipated Study Milestones	**Planned Date**
Start of data collection	Study start will depend on the launch date of *Rotarix* PCV-free liquid vaccine in the participating countries.
End of data collection	Study duration is expected to be 2 years from start of data collection.
End of study	End of study is defined as Completion date of statistical analysis.
Final report of study results	CSR will be submitted to CBER within 1 year of the end of the study.

7. RATIONALE AND BACKGROUND

Rotavirus is the main cause of acute gastro-enteritis and severe diarrhea among young children (<5 years old) in both developing and developed countries; however, there are marked differences in the burden of RV disease, with highest mortality seen in low- and middle- income countries. RV vaccines have been shown to have significant positive impact on diarrheal morbidity and mortality in diverse geographies [WHO, 2021].

Since the introduction of RV vaccine into NIP, the number of deaths from RV in children (<5 years old) has declined by nearly 60% in approximately 10 years with an estimated 122 000 to 215 000 child deaths observed annually during the period 2013 to 2017 compared to 528 000 deaths in 2000. In 2017, RV infection has been estimated to cause 185 300 deaths worldwide among children (<5 years old), nearly all in low- and middle-income countries [ROTA council, 2020; WHO, 2021].

Although RV vaccines are generally well tolerated, they have been associated with a transient increase in the risk of IS. This condition is defined as an acute intestinal obstruction occurring when 1 segment of bowel becomes enfolded within another segment (often near ileocecal junction). IS occurs in young children and is the most frequent cause of an acute abdominal emergency in the first 2 years of life. The peak incidence of IS occurs between 4 and 7 months of age with an incidence which varies broadly across regions. Worldwide, the mean incidence of IS for the period of 2002 to 2012 was 74 per 100 000 person-years (ranging between 9 and 328) among children of less than 1 year of age [Jiang, 2013]. In the LATAM region, IS incidence rate in infants is estimated to range from 3.8 per 100 000 subjects in Brazil to 105.3 per 100 000 subjects in Argentina (Table 1).

Table 1 Annual Incidence Rate of Definite IS (in children <1 year of age) for 11 LATAM Countries with the Highest Incidence

Rank	Country	Incidence of IS in Infants (<1 year old)/100,000
1	Argentina	105.3
2	Mexico	87.8
3	Panama	69.4
4	Guatemala	58
5	Chile	47
6	Dominican Republic	37.8
7	Colombia	37.4
8	Honduras	30.4
9	Peru	25.1
10	Nicaragua	19.6
11	Brazil	3.8

Source: [Sáez-Llorens, 2013; Desai, 2012]

The RRV-TV, *RotaShield*, Wyeth was the first vaccine to prevent RV gastro-enteritis approved for use in the US in October 1998 and was withdrawn less than a year later due to the occurrence of IS as an adverse reaction. Currently, 4 oral live-attenuated, RV vaccines are pre-qualified by the WHO [WHO(a), 2021]. Pre-licensure clinical studies for the 4 RV vaccines did not indicate an increased risk of IS while several post-licensure studies have shown a transient increased risk of IS during the first 7 days following administration of dose 1 of *Rotarix* and *RotaTeq* and, to a lesser extent, after dose 2. The relative risk of IS was estimated at 5.4 (95% confidence interval [CI]: 3.9, 7.4) after dose 1 and 1.8 (95% CI: 1.3, 2.5) after dose 2 of *Rotarix* vaccine within a 7-day period considering a fixed-effect model [Rosillon, 2015]. Post-licensure evaluations of *Rotavac* in India did not indicate an increased risk of IS. For *Rotasiil*, post-licensure safety evaluations have not yet been completed [WHO, 2021].

The lyophilized formulation of *Rotarix* has been licensed in the US since April 2008. In March 2010, health authorities worldwide (including EMA WHO and the US FDA) were informed by GSK of the unexpected presence of PCV type 1 (PCV-1) material in *Rotarix* vaccine. Following the review of the information and data submitted, several of the Health Authorities, including US FDA, EMA and WHO, confirmed that the presence of PCV-1 did not represent a safety risk to the patients. Nevertheless, GSK committed to develop a *Rotarix* PCV-free vaccine (no detection of PCV-1 and PCV-2 according to the limit of detection of the tests used).

As part of the requirements for licensure of *Rotarix* PCV-free liquid vaccine in US, CBER requested GSK to conduct a post -marketing study to assess the risk of IS post-vaccination.

In 2014, GSK performed a preliminary feasibility assessment to conduct the post-marketing study in the US. The enrollment of a sufficient number of subjects to meet the study objectives would require more than 15 years for 3 potential designs (case-control, self-controlled case series, cohort). Upon further discussion with CBER, LATAM countries were identified as more suitable to meet real-world safety evidence needs. Those countries were among the first countries to adopt use of RV vaccines in their NIP [Chavers, 2018]. LATAM countries were selected as region to conduct this post-marketing study because of the high uptake of *Rotarix* vaccine, the incidence of IS and the estimated numbers of subjects to be vaccinated with PCV-free liquid vaccine that could be included in the study.

8. RESEARCH QUESTION AND OBJECTIVES

To evaluate post-authorization, the risk of definite IS following immunization with *Rotarix* PCV-free liquid vaccine in infants less than 1 year old in LATAM.

8.1. Primary objective

To assess the risk of definite IS within 7 days following immunization with the first dose of *Rotarix* PCV-free liquid vaccine.

Refer to Section 9.2.5.1 for the definition of definite IS.

Refer to Section 9.1.3 for the definition of the primary endpoint (see Table 2).

8.2. Secondary objectives

- To assess the risk of definite IS within 7 days following immunization with the second dose of *Rotarix* PCV-free liquid vaccine.
- To assess the risk of definite IS during 3 risk windows following immunization with the first dose of *Rotarix* PCV-free liquid vaccine accounting for time-varying confounders.

Refer to Section 9.2.5.1 for the definition of definite IS.

Refer to Section 9.1.3 for the definition of the secondary endpoints (see Table 2).

8.3. Estimand framework

Table 2 presents the estimands of the study.

Table 2 Endpoint and Estimands

Objective	Endpoints/ Estimands
Primary Objective	
To assess the risk of definite IS within 7 days following immunization with the first dose of *Rotarix* PCV-free liquid vaccine	**Population:** Infants (6 weeks to <1 year of age). Cases are infants hospitalized with definite IS that are at least 6 weeks to <1 year old at the time of diagnosis and controls will be children visiting or hospitalized in the same recruiting hospital as the case, on the same day (+/- 7 days) and matched per age. **Endpoint:** Number of cases (and of controls, separately) exposed to a first dose of RV vaccination within 7 days prior to index date. **Treatment condition:** Immunization with the first dose of *Rotarix* PCV-free liquid vaccine in the 7 days prior to index date. **Summary measure:** Odds ratio of vaccine exposure between cases and controls using a conditional logistic regression model. **Strategy for ICE**: – Co-administration of RV vaccine with OPV for first and second dose: Principal stratum strategy where target population for the

Objective	Endpoints/ Estimands
	analysis is the subset of subjects composed of patients where co-administration of OPV and RV vaccine did not occur.
Secondary Objectives	
To assess the risk of definite IS within 7 days following immunization with the second dose of *Rotarix* PCV-free liquid vaccine	**Population:** Same as primary **Endpoint:** Number of cases (and of controls, separately) exposed to a second dose of RV vaccination within 7 days prior to index date. **Treatment condition:** Immunization with the second dose of *Rotarix* PCV-free liquid vaccine in the 7 days prior to index date. **Summary measure**: Same as primary **Strategy for ICE**: Same as primary
To assess the risk of definite IS during 3 risk windows following immunization with the first dose of *Rotarix* PCV-free liquid vaccine accounting for time-varying confounders	**Population:** Infants (6 weeks to <1 year of age) vaccinated with RV. Definition of cases and controls are the same as primary. **Endpoint:** Occurrence of IS during one of the four intervals: Day 1 to 7, Day 8 to 14, Day 15 to 21, and Day 22 to 35 after first dose of RV vaccine among vaccinated subjects. **Treatment condition:** Immunization with the first dose of *Rotarix* PCV-free liquid vaccine. **Summary measure:** Incidence risk ratio for definite IS using a Poisson regression comparing the one of the risk windows (Day 1 to 7, Day 8 to 14, Day 15 to 21) to the control window (Day 22 to 35). **Strategy for ICE**: Same as primary

ICE: intercurrent events; IS: intussusception; LATAM: Latin America; OPV: oral polio vaccine; PCV: Porcine Circovirus; RV: Rotavirus

9. RESEARCH METHODS

9.1. Study design

9.1.1. Overall design

This study aims to evaluate post-authorization risk of definite IS following immunization with *Rotarix* PCV-free liquid vaccine in infants less than 1 year old in 5 to 8 countries in LATAM. It is a hospital-based, observational, multicenter, prospective, matched case-control study.

Participating hospitals will screen for eligible subjects at least 6 weeks old and less than 1 year of age from daily admission records. Approximately 229 cases and 1374 controls will be enrolled in this study. Cases will include subjects hospitalized or visiting the ER at the time of definite IS diagnosis. Controls will include subjects who are hospitalized or visiting the hospital outpatient clinic or the ER (Refer to Section 9.2.5 for case and control definition). For each case, 6 controls with no gastro-intestinal symptoms (including gastro-enteritis and IS) in the same participating hospital, on the same day as the case (±7 days), will be included after matching for other confounding factors (Refer to Section 9.2.3 for matching criteria).

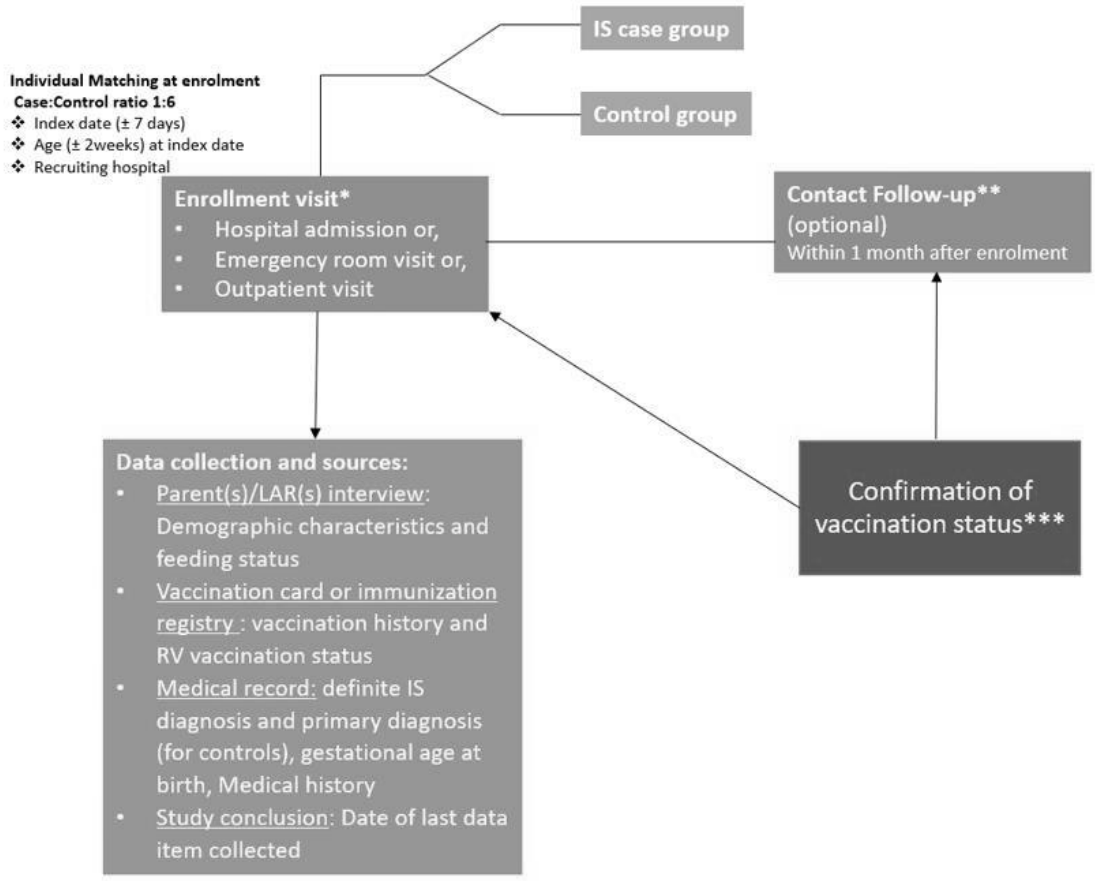
At the enrollment visit, eligible subjects (case/ control) will be included in the study after completion of a written informed consent from the parent(s) and/or LAR(s) (according to local regulatory requirements). The parent(s) and/or LAR(s) will be interviewed to gather information such as demographic characteristics (e.g., date of birth, gender, race and/or ethnicity) and feeding status. For both cases and controls, the investigator will review the vaccination card in order to ascertain vaccination history (including dates of vaccination, number of doses [e.g., 1, 2] administered, brand name [for the RV vaccines] and batch number [only for *Rotarix*]) either during enrollment visit or through a contact follow-up with parents and/or LAR(s). If the vaccination card cannot be provided, the study team will try to obtain information from immunization registries. Medical records will be reviewed to gather information on IS diagnosis procedures and final diagnosis, gestational age at birth, and medical history. This is a self-contained study where appropriate information mentioned above will be reported in an eCRF after reviewing the medical records, vaccination cards and immunization registries for information accuracy.

Subjects' enrollment will be confirmed when the vaccination status is known. For both cases and controls, the investigator will review the vaccination cards to confirm whether any RV vaccine or poliomyelitis vaccines were administered to the child. The review of vaccination cards will be conducted either during hospitalization or during visit to the hospital outpatient clinic/ER. When the vaccination status cannot be ascertained at the time of enrollment visit, a contact follow-up with the parent(s) and/or LAR(s) will take place within a month after the enrollment visit. At least 3 attempts will be made to contact the parent/LAR(s) before the subject is determined as lost to follow-up. If vaccination status is still not available at the contact follow-up, subject will be withdrawn from the study.

A screening log of all potentially eligible cases and controls who did not meet final eligibility criteria will be maintained on site, so that reasons for exclusion or non-participation can be recorded.

The study schematic is shown below in Figure 1.

Figure 1 Study Design Overview



ER: Emergency Room; IS: Intussusception, LAR(s): Legally Acceptable Representative (s), RV: Rotavirus
*Eligible cases will be selected among subjects hospitalized or visiting the ER at the time of diagnosis of definite IS while controls will include subjects who are hospitalized or visiting the hospital outpatient clinic or the ER. For each case, 6 controls with no gastro-intestinal symptoms (including gastro-enteritis and IS), will be included after matching for other confounding factors.
**When the vaccination status cannot be ascertained at the time of enrollment visit, a contact follow-up with the parent(s) and/or LAR(s) will take place within a month after the enrollment visit (at least 3 attempts will be made to contact the parent(s) and/or LAR(s) before the subject is determined as lost to follow-up).
***If vaccination status is still not available at the contact follow-up, subject will be withdrawn from the study.

Details of data collection are summarized in Table 4.

9.1.2. Scientific rationale for study design

Briefly, a prospective, matched case-control study design was chosen to optimize data collection for a very rare disease such as IS where a cohort study would require a long period of follow-up and a larger sample size to meet the study objectives. Another advantage of the case-control design is that existing immunization registries can be used to ascertain vaccination status.

This is a multicenter hospital-based study where cases and controls will be recruited in the same hospital during the same period. Additionally, the enrollment of 6 controls per case contributes to increase the statistical power of the study by increasing the sample size. Finally, variability is allowed in age for matching controls to cases (±2 weeks) and in index date (±7 days) to facilitate enrollment.

9.1.3. Study endpoints

Primary endpoint: Number of cases (and of controls, separately) exposed to a first dose of RV vaccination within 7 days prior to index date.

Secondary endpoints:

- Number of cases (and of controls, separately) exposed to a second dose of RV vaccination within 7 days prior to index date.
- Occurrence of IS during one of the four intervals: Day 1 to 7, Day 8 to 14, Day 15 to 21, and Day 22 to 35 after first dose of RV vaccine among vaccinated subjects.

The index date will be defined as the onset date of the definite IS episode for cases and the hospital admission date or outpatient or/ ER visit date for controls.

9.1.4. Study duration

The duration of the study will be for a period of at least 2 years from the date of FSFV and, if needed, extended to ensure enrollment of all cases and controls required to reach the targeted sample size (Table 3). In each study country, the study start will depend on (1) *Rotarix* PCV-free liquid vaccine launch date and (2) market share at that time. Refer to Section 9.5 for details about sample size and duration of enrollment.

Table 3 Study Groups Foreseen in the Study

Study Groups	Number of Subjects	Age (Min/Max)
Cases	229	6 weeks/<1 year
Controls	1374	6 weeks/<1 year

- PCD: Date of final collection of data for all primary outcomes/endpoints.
- EoS: Date of completion of statistical analysis.

Refer to glossary of terms (Annex 2) for the definition of PCD and EoS.

9.1.5. Strength of the study design

As described in Section 9.1.6, a feasibility assessment was conducted to determine the most suitable countries for subject recruitment.

Launch date and implementation of *Rotarix* PCV-free liquid will be verified for each country closer to study start and could affect the final choice of country selection.

Only confirmed cases of definite IS (as per Level 1 of diagnosis certainty in Brighton Collaboration IS Working Group definition) will be considered for analysis. This increases the specificity of the study endpoint and reduces possible bias to the null hypothesis.

The odd ratio of IS will be estimated separately after the first and second dose of the *Rotarix* PCV-free liquid vaccine, which provides an indication of the vaccine's safety profile after administration of each dose.

Another key strength of the study design resides in the confirmation of the vaccination status upon enrollment. All subjects for whom vaccination status cannot be ascertained (through consultation of vaccination card or immunization registry and after adequate contact follow-up as per study protocol) will be withdrawn from the study.

9.1.6. Summary of study feasibility

To assess the feasibility of this post-marketing study, a comprehensive feasibility approach was performed in 2024, to evaluate potential designs and countries. The list of candidate countries for this feasibility assessment was restricted by applying the following pre-specified criteria:

a. Background incidence of IS >25 per 100 000 person-years [Sáez-Llorens, 2013]

b. RV vaccination coverage >70% [PAHO(a), 2022]

c. *Rotarix* is currently recommended in the immunization schedule of the country.

Countries that met at least 2 of the 3 criteria listed above were further considered for the operational feasibility. An investigator site outreach was conducted by sending a detailed site questionnaire to 88 investigators from 9 countries (Argentina, Colombia, Costa Rica, Dominican Republic, Guatemala, Honduras, Mexico, Panama, and Peru). Feedback was received from 49 investigators along with medical and operational inputs from their study team. This information was included in the assessment of the overall study strategy. The selection process returned 8 candidate countries for the study: Argentina, Colombia, Costa Rica, the Dominican Republic, Guatemala, Honduras, Mexico, and Panama. To allow for the recruitment of the calculated sample size within the estimated 24 months for participant recruitment, a minimum of 5 countries will be selected. The number of countries can be extended up to 8, if recruitment does not reach the estimated enrollment projections.

9.2. Setting

9.2.1. Study population

The source population for this study will be infants (6 weeks to <1 year of age at the time of IS diagnosis) visiting or hospitalized in a recruiting hospital and who meet the study eligibility criteria at the time of entry into the study. The target enrollment will be 1603 subjects (229 IS and 1374 controls with a ratio of 6 controls per case). Section 9.2.3 provides a detailed definition of cases, controls, and matching criteria.

9.2.2. Overview of recruitment plan

All subjects will be identified in participating hospitals across 5 to 8 countries in LATAM. Within each participating country a target number of hospitals will be selected to be part of this study based on sites identified in the feasibility assessment performed in 2024.

Cases will include all infants hospitalized or visiting the ER for whom a definite IS diagnosis is established at the participating hospitals during the study period, and aged at least 6 weeks old and less than 1 year of age at the time of diagnosis of IS. Cases will be identified by periodic reviews of pediatric ward admission and ER logbook(s). For each eligible case enrolled in the study, 6 matching controls will be identified according to the matching factors described in Section 9.2.3.1. Eligible controls will be identified during their visit to the recruiting hospitals. The investigator should make every effort to select suitable age-matched controls as soon as a case is identified.

If a child meets the eligibility criteria at the enrollment visit, his/her parent(s) and/or LAR will be invited to consent to the subject participating in the study. Following enrollment, subjects will be given a unique subject identifier.

9.2.3. Subject selection criteria

9.2.3.1. Inclusion criteria for enrollment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study or regulatory acceptability. Therefore, adherence to the criteria as specified in the protocol is essential.

9.2.3.1.1. Inclusion criteria for enrollment of IS cases

All IS cases must satisfy ALL the following criteria at study entry:

1. A male or female subject aged at least 6 weeks old and less than 1 year at the time of diagnosis of definite IS (subjects are ineligible before 6 weeks of age and as of their first birthday).
2. Subject hospitalized or visiting the ER of the participating hospitals during the study period for definite IS, according to the guidelines from the Brighton Collaboration IS Working Group, (Refer to Section 9.2.5.1).
3. Subjects whose parent(s)/LAR(s), in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., agree to provide vaccination status during contact follow-up if not available at enrollment visit).
4. Subject whose parent(s)/LAR(s), provide written informed consent (according to applicable local regulations).

9.2.3.1.2. Inclusion criteria for enrollment of controls

All control subjects must satisfy ALL the following criteria at study entry:

1. A male or female subject aged at least 6 weeks old and less than 1 year at the time of hospitalization or visit to the hospital outpatient clinic or ER (subjects are ineligible before 6 weeks of age and as of their first birthday).
2. Subject hospitalized or visiting the hospital outpatient clinic or the ER of the participating hospitals during the study period with no gastro-intestinal symptoms (including gastro-enteritis and IS).
3. Subjects whose parent(s)/LAR(s), in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., agree to provide vaccination status during contact follow-up if not available at enrollment visit).
4. Subject whose parent(s)/LAR(s), provide written informed consent (according to applicable local regulations).

Matching factors

Six controls will be matched to each case according to the following factors:

- Index date[1] (±7 days)
- Age (weeks) at index date (±2 weeks)
- Recruiting hospital

[1] The index date will be the onset date of the definite IS episode for cases and the hospital admission date or outpatient/ER visit date for controls.

9.2.3.2. Exclusion criteria for enrollment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study or regulatory acceptability. Therefore, adherence to the criteria as specified in the protocol is essential.

9.2.3.2.1. Exclusion criteria for enrollment of IS cases

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the IS case must not be included in the study:

5. Subject has previously participated as a case or a control in this study, either in the same hospital or in another participating hospital.
6. Subject is enrolled in another clinical trial.

9.2.3.2.2. Exclusion criteria for enrollment of controls

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the control must not be included in the study:

1. Subject has previously participated as a case or a control in this study, either in the same hospital or in another participating hospital.
2. Subject is enrolled in another clinical trial.
3. Subject has gastro-intestinal symptoms (including gastro-enteritis and IS) during current hospitalization/visit to the hospital outpatient clinic or ER, at the time of enrollment visit.

9.2.4. Subject withdrawal

Parents withdrawing their consent to participate to the study will be withdrawn from the study at any time and without any impact on the care they receive. Whenever possible and irrespective of the reason for withdrawal, the date and reason for withdrawal should be recorded in the eCRF. If a patient discontinues due to withdrawal of consent, no new data should be collected after the withdrawal of consent.

Subjects for whom the RV vaccination status was not ascertained at enrollment and could not be ascertained during subsequent follow-up contact, will be considered as discontinued and excluded from the analysis.

Intussusception (IS) cases enrolled in the study and not confirmed by the adjudication will be considered as Screening Failures. These cases will be excluded from the analysis and will not contribute to the sample size calculation defined in Section 9.5. If these cases had matching controls enrolled in the study, these controls will be discontinued.

9.2.5. Subject definitions

9.2.5.1. Cases

The following criteria will be used to identify definite IS cases. This is based on the definitions of acute IS in the guidelines from the Brighton Collaboration IS Working Group[Bines, 2004]. A definite IS case corresponds to Level 1 of diagnostic certainty.

Level 1 of diagnostic certainty:

Surgical:

- The demonstration of invagination of the intestine at surgery,

And/or

Radiological:

- The demonstration of invagination of the intestine by either air or liquid contrast enema or
- The demonstration of an intra-abdominal mass by abdominal ultrasound with specific characteristic features (target sign or doughnut sign on transverse section and a pseudo-kidney or sandwich sign on longitudinal section) that is proven to be reduced by hydrostatic enema on post-reduction ultrasound

And/or

Autopsy:

- Intestinal invagination observed at autopsy

Refer to Section 9.2.3 for inclusion/exclusion enrollment criteria for IS cases.

Definite IS cases included in the study will undergo a thorough adjudication process to ensure accuracy and consistency in the evaluation of the event. The process will be described in a clinical event committee charter.

9.2.5.2. Controls

Subjects will be selected according to the matching criteria defined in Section 9.2.3.

9.2.5.3. Exposure (*Rotarix* PCV-free liquid vaccination)

A subject will be considered as exposed if immunization with the *Rotarix* PCV-free liquid vaccine occurred within 7 days before the index date (i.e., on the previous 7 days before the index date).

9.2.6. Study assessments and data collection

All the information about subjects enrolled at each hospital will be collected from paper or EMRs, parent(s)/LAR(s) interview, as well as vaccination cards and immunization registry as allowed by local regulations. The study-related information gathered through these sources will be entered into an eCRF (further details are provided in [Section 9.6])

Demographic characteristics (e.g., date of birth, gender, race and/or ethnicity) and feeding status will be provided by the parent(s) and/or LAR(s) during an interview. The vaccination history and RV vaccine status will be ascertained by the investigator based on the vaccination card or retrieved from immunization registries. Medical records will be reviewed to gather information on IS diagnostic procedures and final diagnosis for cases, primary diagnosis for controls, medical history gestational and age at birth.

The data collection schedule is presented in Table 4

Table 4 Data Collection Schedule

Age	**6 weeks – 12 months of age**	**<13 months of age**
Time points	Enrollment visit (hospitalization or outpatient clinic visit or ER visit)	Contact Follow-up (*optional) within 30 days after enrollment
Informed consent	X	
Inclusion/Exclusion criteria	X	
Parent(s)/LAR(s) interview and review of medical records to collect information in the eCRF on:		
Demographic characteristics - date of birth, - gender, - Race and / or ethnicity (where feasible according to local regulation)	X	
Feeding status (exclusive breastfeeding, formula feeding, mixed feeding)	X	
Medical history - gestational age at birth - history of IS - history of gastro-enteritis (viral/bacterial) in the previous 30 days - history of enema - uncorrected condition of the gastro-intestinal tract	X	
Vaccination history and concomitant medication: – Vaccination against RV: • date[s] of vaccination, • dose number (e.g., 1, 2), • brand name of the vaccine • for *Rotarix* only: batch number – Vaccination with OPV and IPV • date[s] of vaccination and dose number [e.g., 1, 2] as applicable, i.e. according to the schedule of vaccination in each	X	X

country, including when co-administrated with RV vaccine		
Diagnostic procedures and final diagnosis: – Date of definite IS – Confirmation of diagnosis of definite IS for cases – Primary diagnosis for controls – Index date	X	
Adjudication process: – All clinical signs and symptoms and diagnosis procedures and outcomes to confirm the IS diagnosis as described in the clinical event committee charter diagnosis	At enrollment and at any timepoint during the study	
Study conclusion – Date of last data item collected for the subject	X	X**

eCRF:electronic Case Report Form; ER:Emergency room; EMR:Electronic medical records; IS: Intussusception; LAR: Legally Acceptable Representative; RV: Rotavirus; OPV: Oral polio vaccine; IPV: Inactivated poliovirus vaccine.
X indicates the expected set of variables that will be documented in the eCRF.
Note: For this study, demographic characteristics and feeding status will be reported by parent(s)/LAR(s) while all other information will be collected from the EMR or paper medical record, vaccination card or immunization registries.
* A contact follow-up within a month after enrollment visit will allow the collection of vaccination history when vaccination history cannot be ascertained at enrollment visit.
** Collected for subjects for whom all information was not available at enrollment.

9.3. Variables

9.3.1. Variables collected at enrollment

1. **Demographic characteristics**
   - Date of birth
   - Gender
   - Race and/or Ethnicity (where feasible according to local regulation)
2. **Case – control linkage**
   - Site Id
   - Matching case Id for controls
3. **Feeding status** (exclusive breastfeeding/formula feeding/mixed feeding)
4. **Medical history**
   - Gestational age at birth (in weeks)
   - History of IS
   - History of gastro-enteritis (viral/bacterial)
   - History of enema
   - Uncorrected condition of the gastro-intestinal tract
5. **Vaccination history**

- Vaccination against RV
  - Date[s] of vaccination
  - Dose number (e.g., 1, 2)
  - Brand name of the vaccine
  - Batch number (for *Rotarix* only)
- Vaccination with OPV or IPV *(including vaccine co-administrated with RV vaccine)* as applicable according to the schedule of vaccination in each country
  - Date[s] of vaccination
  - Dose number (e.g., 1, 2) if applicable

6. **Diagnostic procedures and final diagnosis**
   - Date of definite IS
   - Confirmation of diagnosis
     - Definite IS for cases (Level 1 of diagnostic certainty as per Brighton Collaboration IS Working Group definition).
     - Primary diagnosis for controls
     - All clinical signs and symptoms and diagnosis procedures and outcomes to confirm the IS diagnosis as described in the clinical event committee charter (at enrollment and any time point during the study)
   - Index date (diagnosis date of definite IS for case or the hospital admission date or outpatient/ER visit for control)
7. **Study conclusion**
   - Date of last data item collected for the subject

9.3.2. Variables collected during contact follow-up *(when not available at enrollment visit)*

1. **Vaccination history**
   - Vaccination against RV
     - Date[s] of vaccination
     - Dose number (e.g., 1, 2)
     - Brand name of the vaccine
     - Batch number (for *Rotarix* only)
   - Vaccination with OPV or IPV vaccine *(including vaccines co-administrated with RV vaccine)* as applicable according to the schedule of vaccination in each country

- Date[s] of vaccination
- Dose number (e.g., 1, 2) if applicable

2. **Diagnostic procedure and final diagnosis**

- Confirmation of diagnosis
  - All clinical signs and symptoms and diagnosis procedures and outcomes to confirm the IS diagnosis as described in the clinical event committee charter (at any time point during the study)

3. **Study conclusion**

- Date of last data item collected for the subject

9.4. Data sources

The data for this study will be retrieved by trained site personnel from electronic or paper medical records, vaccination cards, immunization registries and parent(s)/LAR(s) interviews completed by the investigator or designee. Data will be entered directly into the database through web-based eCRFs in each participating study-site. Sites will be asked to collect relevant clinical data only for eligible subjects. Study-specific data will be transmitted in a secure manner to GSK or GSK's representative CRO.

Data must be entered into the eCRF in English. The eCRF must be completed as soon as possible after an enrollment visit and/or contact follow-up visit, and the forms should be available for review at the next scheduled monitoring visit. If necessary, queries will be generated in the EDC platform.

- Investigator and study-site personnel can make corrections in the EDC platform at their own initiative or as a response to an auto query (generated by the EDC platform).
- GSK or GSK's representative can generate a query for resolution by the investigator and study-site personnel.

9.5. Study size

9.5.1. Sample size for primary analysis (case-control design)

Several parameters, derived from national vaccination recommendations, currently available data on local vaccine coverage, IS disease epidemiology, as well as *Rotarix* vaccine market share in targeted countries (when available), have been considered to compute the sample size.

The expected proportion of controls exposed to RV vaccination (Table 7) was computed from:

1. The age distribution of IS cases in the US derived from Tate et al.[Tate, 2008].Table 5
2. The age distribution at *Rotarix* vaccination in the US derived from the GSK-sponsored study EPI-ROTA-007 VS US DB (112229) (Table 6)

For a 100% RV vaccination coverage:

- % exposed controls (dose 1/2) in Month i = (% of exposed subjects vaccinated in month i) x (% of IS cases occurring in month i)
- % exposed controls (dose 1/2) in Week i =% exposed controls (dose 1/2) in Month i *(7/30.5)
- Example for % exposed controls in one week of the age category]2-3] months: (74.9% x 3.82%)*(7/30.5)=0.657%.

According to WHO vaccine-preventable diseases monitoring system [WHO(b), 2022], estimates for RV vaccination coverage in 2022 varied from 84.6% to 97.7% for the first dose in the 5 pre-selected countries. The coverage for the second dose of RV vaccine ranges between 79.2% and 85.9%. The vaccination coverage for the first dose used for the sample size calculation was an estimate of 90%. In addition, since the vaccine product used in the NIP is *Rotarix* for the targeted countries, a conservative estimate of 90% market share was applied [International vaccine access center, 2022]

The rate of exposure in controls for each week was then adjusted for those estimates:

- Adjusted % exposed controls (dose 1/2) in Week i=[% exposed controls (dose 1/2) in Week i *(% market share * %vaccination coverage)]/[(100%- %vaccination coverage)+(% market share * % vaccination coverage)]
- Example for adjusted % exposed controls in 1 week of the age category [2-3] months: 0.657%*(90%*90%)/[(100%-90%)+(90%*90%)]=0.585%

The expected proportion of subjects exposed to *Rotarix* within 1 week before IS was computed as the sum of the rates of exposure in one week for each age category (month) below 1 year of age. This resulted in an estimated overall rate of exposure in controls of 0.790% for the first dose and or 2.055% for the second dose (Table 8).

Table 5 Age (in months) Incidence of IS in Children Less than 1 Year in the US Population

Age (month)	N (case/100 000)	%
[0-1]	0.2	0.59
]1-2]	0.6	1.76
]2-3]	1.3	3.82
]3-4]	2.4	7.06
]4-5]	3.5	10.29
]5-6]	4.4	12.94
]6-7]	4.6	13.53
]7-8]	4.3	12.65
]8-9]	4.0	11.76

Age (month)	N (case/100 000)	%
]9-10]	3.6	10.59
]10-11]	2.9	8.53
]11-12]	2.2	6.47
Total	**34**	**100**

IS, intussusception; US, United States; N, number.
Source: [Tate, 2008]

Table 6 Age Distribution (in months) at *Rotarix* Vaccination in the US Population

Age (month)	Dose 1 (%)	Dose 2 (%)
[0-1]	0.0	0.0
]1-2]	17.7	0.0
]2-3]	74.9	0.5
]3-4]	2.8	12.9
]4-5]	3.8	78.9
]5-6]	0.3	4.4
]6-7]	0.4	2.9
]7-8]	0.1	0.3
]8-9]	0.0	0.1
]9-10]	0.0	0.0
]10-11]	0.0	0.0
]11-12]	0.0	0.0

US: United States.
Derived from data of the GlaxoSmithKline Biologicals, SA (GSK) sponsored EPI-ROTA-007 VS US DB (112229) study.

Table 7 Expected Proportion of Controls Exposed for 100% Rotavirus Vaccination Coverage*

Age (months)	% of Exposed controls per week (Dose 1)	% of Exposed controls per week (Dose 2)
[0-1]	0.000	0.000
]1-2]	0.072	0.000
]2-3]	0.657	0.004
]3-4]	0.045	0.209
]4-5]	0.090	1.864
]5-6]	0.009	0.131
]6-7]	0.012	0.090
]7-8]	0.003	0.009
]8-9]	0.000	0.003
]9-10]	0.000	0.000
]10-11]	0.000	0.000
]11-12]	0.000	0.000
Total	**0.888**	**2.310**

US: United States
*Based on incidence of IS per age category (in months) and age distribution (in months) at *Rotarix* vaccination in children less than 1 year in the US population.

Table 8 Expected Proportion of Controls Exposed Adjusted for 90% Rotavirus Vaccine Coverage and 90% Market Share of *Rotarix*

Age (months)	% of Exposed Controls (Dose 1)	% of Exposed Controls (Dose 2)
[0-1]	0.000	0.000
]1-2]	0.064	0.000
]2-3]	0.585	0.004
]3-4]	0.040	0.186
]4-5]	0.080	1.659
]5-6]	0.008	0.116
]6-7]	0.011	0.080
]7-8]	0.003	0.008
]8-9]	0.000	0.002
]9-10]	0.000	0.000
]10-11]	0.000	0.000
]11-12]	0.000	0.000
Total	**0.790**	**2.055**

Table 9 Sample Size for a Case-Control Design

Objective	Odds Ratio	% Exposed in Controls	Number of Cases Needed with 6 Controls Per Case	Number of Cases Needed with 4 Controls Per Case
Primary (Dose 1)	5.4	0.790	199	229
Secondary (Dose 2)	5.4	2.055	81	93

Sample size calculation to test for 2 correlated proportions in a matched case-control design with a 0.2 correlation, performed using PASS 2021, v21.0.3 A 1-sided type 1 error of 2.5% and a power of 80% was also used. Assumptions were leveraged from a prior study [Dupont, 1988]

Based on the sample size calculation and considering a RV vaccine coverage of 90% (with 90% market share for *Rotarix*) in pre-selected countries, 199 IS cases with 6 controls per case would be needed to detect a risk of IS of 5.4 after the first vaccine dose (Table 9).

To consider potential subjects who have received another RV or OPV vaccine, the number of subjects to be enrolled will be increased by 15%. **Therefore, approximately 229 cases and 1374 controls will need to be enrolled.**

The number of controls included per case will be closely monitored during the recruitment period. If the targeted number of 6 controls per case cannot be reached, the sample size estimation will be adapted. To conserve the same power and account for potential subjects who have received another RV or OPV vaccine, 35 additional cases will be recruited to match 1056 controls (for a 1:4 case/control ratio). If needed, the study duration will be extended to allow the enrollment of the additional cases.

All calculations were performed with a 80% power to reject the null hypothesis: no increased risk of IS during the 7 days after vaccination with the first dose of *Rotarix*

PCV-free liquid vaccine, if the true odds ratio is 5.4 (estimated risk with *Rotarix* vaccine after dose 1 [Rosillon, 2015]).

9.5.2. Sample size for SCRI analysis

Table 10 provides the number of cases needed in the analysis set for SCRI analysis to reach 80% power to detect a risk increase while controlling the one-sided type I error to 2.5%.

Table 10 Number of Cases Needed in the Analysis Set for SCRI Analysis to Reach 80% Power to Detect a Risk Increase While Controlling the One-Sided Type I Error to 2.5%

Dose 1		Dose 2	
Odd Ratio	**Number of Cases**	**Odd Ratio**	**Number of Cases**
5.4	12	1.8	95
10.8	7	3.6	20
27.0	4	9.0	8

SCRI: Self-Controlled Risk Interval

Note: The ratio of the length of the risk period over the observation period used in the calculations was 0.33 (i.e., the risk period of 7 days divided by the overall observation period of 21 days including 1 week of risk period and 2 weeks of control period).

Method: [Musonda, 2006]

9.6. Data management

To optimize quality and accelerate data collection, an eCRF (web-based form) will be used for collection of demographic and clinical data, utilizing a 21 CFR Part 11 compliant EDC platform.

Study sites will be provided with instructions to assist with accurate and consistent retrieval of the data from the medical charts. The same eCRF will be used by each participating hospital. Structured training related to data entry and a pilot test of the eCRF will be conducted for each center, so that chart abstraction for the eCRF is conducted in accordance with the study protocol.

A data management plan will be created before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning and validation. The eCRFs will include programmable edit checks to obtain immediate feedback, if data are missing, out of range, illogical or potentially erroneous. Concurrent manual data review will be performed based on parameters dictated by the plan. Ad hoc queries will be generated within the EDC system and followed up for resolution. High data quality standards will be maintained, and processes and procedures utilized to repeatedly ensure that the data are fully cleaned when presented for analysis.

9.7. Data analysis

9.7.1. General considerations

The statistical analyses described in this section will be detailed in a SAP.

Analysis and generation of tables, listings and figures will be performed using statistical analysis system (SAS®) version 9.4 or higher (SAS Institute, Cary, NC, USA).

Demographic characteristics, feeding status, medical history and vaccination history will be summarized for all subjects and separately for cases and controls.

IS diagnosis procedures and final diagnosis will be summarized for cases and primary diagnosis will be summarized for controls.

Handling of missing data values will be described in the SAP.

9.7.2. Analysis datasets

9.7.2.1. Analysis dataset for the case-control analysis

The dataset for the case-control design will include all subjects enrolled in the study for whom RV vaccination status is known.

Intussusception cases and controls with confirmed administration of a RV vaccine other than *Rotarix* PCV-free liquid vaccine will be excluded from the analyses. In addition, subjects with OPV co-administration will be excluded from the primary analysis.

This study population will include definite IS cases and control subjects.

9.7.2.2. Analysis dataset for the SCRI analysis

The SCRI analysis will use only vaccinated cases occurring in pre-specified risk or control intervals “windows”. Each subject will serve as its own control, thus controlling for fixed potential confounders, whether they are known or not. In addition, in using only vaccinated cases the SCRI design will avoid the bias that can affect cohort studies when vaccinated subjects are misclassified as unvaccinated. The null hypothesis will be that the risk of the outcome on an average day during the pre-defined risk intervals after vaccination (Day 1 to 7, Day 8 to 14, and Day 15 to 21) is the same as the risk of the outcome on an average day during the pre-defined control interval.

The dataset for the SCRI design will include cases from the analysis dataset for the case-control analysis for whom definite IS diagnosis occurred either during the risk periods (Day 1 to 7, Day 8 to 14, and Day 15 to 21) after the first dose of *Rotarix* PCV-free liquid vaccine or between 22 to 35 days (control period) after the first dose of *Rotarix* PCV-free liquid vaccine.

9.7.3. Statistical analyses

9.7.3.1. Descriptive analysis

All data collected for the study will be summarized with appropriate statistics. For continuous variables, the mean, standard deviation, median, lower and upper quartiles, minimum and maximum will be provided. For categorical variables, the numbers and percentages will be provided, as well as the associated 95% CI when appropriate. Percentages will be computed among subjects with available data. In addition, the number of missing values will be presented.

Demographic characteristics, medical history, vaccination history will be summarized for all subjects and separately for cases and controls using methods described in the SAP.

IS diagnosis procedures and final diagnosis will be summarized for cases and primary diagnosis will be summarized for controls.

9.7.3.2. Primary endpoint analyses

The case-control analysis will serve as the primary analysis. To estimate the vaccine exposure between cases and controls, a conditional logistic regression model will be used, the matched odds ratio of vaccine exposure between cases and controls, 95% CI, and p-values will be presented.

Gender, race or ethnicity (if available) and feeding status may be included in the model (but may not limited to) as potential confounders and effect measure modifiers as will be described in the SAP.

Subgroup analyses by country will be provided as supportive analysis.

9.7.3.3. Secondary endpoints analyses

Second dose of RV vaccine
The analyses performed for the primary endpoint will be repeated for the second dose (Refer to Section 9.7.3.2).

First dose of RV vaccine: SCRI analysis
To compare the incidence of IS between the risk period and the control period, a Poisson regression stratified by subject will be used and the incidence rate ratio of IS (risk/control), 95% CI, and p-values will be presented. The analysis will only be powered if the number of IS cases exceeds 4 (Refer to Section 9.5.2).

The following periods will be considered:

- Risk periods:

  - Day 1 to Day 7 after first dose of *Rotarix* PCV-free liquid vaccine.
  - Day 8 to Day 14 after first dose of *Rotarix* PCV-free liquid vaccine.
  - Day 15 to Day 21 after first dose of *Rotarix* PCV-free liquid vaccine.
- Control period: Day 22 to Day 35 after first dose of *Rotarix* PCV-free liquid vaccine.

9.7.3.4. Conduct of analyses

A final study report will be written at the end of the study when the data for the entire study period have been collected. No interim analysis is planned.

9.8. Quality control

To ensure accuracy and reliability of the study:

- Qualified investigators and appropriate study sites will be selected,
- Protocol procedures will be reviewed with the investigator and study-site personnel:

1. before the study start,
2. during periodic monitoring visits and,
3. during the transfer of clinical data from EMR into the GSK's database.

Written instructions will be provided to recruiting hospitals for collection, handling, and storage of data. Guidelines for eCRF completion will be provided and reviewed with study-site personnel before the start of the study. All eCRF entries, corrections, and modifications must be made legibly by the investigator or authorized study-site personnel. The investigator must verify that all data entries in the eCRF are accurate and correct. The appropriate pages of the eCRF must be signed and dated by the investigator. The CRO will review the eCRF for accuracy and completeness during monitoring visits and before transfer to GSK.

9.9. Limitations of the research methods

- The feasibility to conduct a post-marketing study on IS after administration of the *Rotarix* PCV-free liquid vaccine in selected countries depends on the use of *Rotarix* in these countries. As in any country, this depends on the public health governance at national level and the willingness to sustain current RV vaccination recommendations.
- As per the preliminary scientific feasibility assessment, in case the study cannot be implemented in the selected countries due to reasons including willingness of the investigators to contribute to the study, operational constraints related to capacity of study sites, switch to another RV vaccine, regulatory variation procedure lead-time, or unexpected decrease in *Rotarix* PCV-free liquid vaccination coverage, there is a risk that the full study cannot be conducted as the sample size would be too limited and/or study duration would be too long.

- Timelines of introduction of the *Rotarix* PCV-free liquid vaccine in each of the targeted countries are closely linked to timings of approvals by regulatory authorities. In addition, GSK is not directly monitoring the distribution of vaccine doses in the pre-selected countries. Most of the countries in the region receive RV vaccine doses through PAHO. GSK cannot guarantee the timing of introduction and full usage of the *Rotarix* PCV-free liquid vaccine. It cannot be excluded that the study will start at different points in time in the different countries, depending on regulatory and operational timelines.
- The study will only be powered for a pooled country approach. Data generated per country will not have sufficient power to detect an increased risk of IS after immunization with *Rotarix* PCV-free liquid vaccine in a given country. The number of subjects to be enrolled in the study is estimated to 229 cases and 1374 controls with a ratio of 6 controls per case. During the recruitment, the number of controls selected per case will be closely monitored and if the targeted number of 6 controls per case cannot be reached, the sample size estimation will be adapted. To conserve the same power, 35 additional cases will be recruited to match 1056 controls (for a 1:4 case/control ratio). If needed, the study duration will be extended to allow the enrollment of the additional cases.
- Administration of OPV together with RV vaccine had been shown to decrease the risk of IS after the first dose of RV vaccine [Patel, 2011]. The first dose of OPV, which is the dose associated with the greatest replication of vaccine poliovirus strains, is known to decrease the immunogenicity of the first dose of RV when these 2 oral vaccines are administered together. Co-administration of the 2 vaccines may thus reduce the risk of IS if this risk correlates with the vaccine immune response. However, following the PAHO recommendation, a switch from OPV to IPV has been initiated in LATAM, starting in 2016 [PAHO(b), 2018]. As of today, all LATAM countries have implemented, as first dose, the use of IPV for children aged less than 1 year of age. Nonetheless, given the limited information on the risk of IS after administration of *Rotarix* in LATAM countries, there is a possibility to not observe an increased risk of IS after RV vaccination.
- SCRI analysis, as recommended by CBER, will be conducted as a secondary endpoint. The analysis will only be powered if the number of IS cases is sufficient.
- Only IS cases and controls with known vaccination status will be included in the analysis. If the information is not available at enrollment, the possibility of a contact follow-up to collect the vaccination status from the vaccination card is considered in the data collection process. However, the compliance with this procedure is uncertain. Challenges in obtaining the vaccination status of eligible subjects, especially the vaccination date, may thus impact the enrollment timelines.

9.10. Other aspects

Not applicable.

10. PROTECTION OF HUMAN SUBJECTS

To ensure the quality and integrity of research, this study will be conducted under GPP issued by ISPE, the Declaration of Helsinki and its amendments, and any applicable local and national guidelines. The study will be conducted in compliance with the US FDA Title 21 CFR Part 50 – Protection of Human Patients and/or Part 56 – IRB; the ICH GCP E6 guideline (revision 2) (15 December 2016) as they apply to post-marketing, observational studies; the Declaration of Helsinki and its amendments and the HIPAA.

Study will start only after favorable opinion/approval to conduct the study is obtained from national regulatory authorities and IRB/IEC in each country, prior to a site initiating enrollment in that country.

Freely given and written or witnessed informed consent must be obtained from each subject's parent(s)/LAR(s) or the impartial witness, as appropriate, prior to participation in the study. Impartial witness shall be present whenever informed consent is provided by parents(s)/LAR(s) who cannot read or write.

A model ICF which will embody the applicable ICH GCP or other applicable guidelines, and Company required elements will be prepared while it is strongly recommended that this model ICF be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgment, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the GSK's representative (CRO) must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

This study is a hospital-based, observational, multicentere, prospective matched case-control post-marketing study, based on data extracted from hospital medical records, parent(s)/LAR(s) interviews, vaccination cards and immunization registries. *Rotarix* PCV-free liquid vaccine will not be administered during the study. Therefore, individual case adverse events (AE)/adverse reaction reports will not be generated from this study.

Healthcare providers who treat the subjects included in this study are encouraged to submit case reports of AEs following any vaccination, including *Rotarix*. Spontaneous reports of SAEs received by GSK are processed according to standard pharmacovigilance procedures, which include reporting of AEs, adverse reactions and SAEs to regulatory authorities.

All safety information collected from the study cases will be gathered and assessed in PBRERs.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

Upon study completion and finalization of the study report, the results of this non-interventional study will be posted in a publicly accessible register (e.g., GSK Clinical Study Register, clinicaltrials.gov). The results will also be submitted for publication to a peer reviewed journal within the policy defined timelines. Publications will comply with internal GSK standards and the ICMJE guidelines.

13. REFERENCES

References can be provided upon request.

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Annex 1 List of standalone documents

No.	Document Reference No	Date	Title
1	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	List of standalone documents
2	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	Glossary of terms
3	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	List of principal and coordinating investigators
4	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	Sponsor information
5	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	Amendment and administrative changes to the protocol
6	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	Additional information
7	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.1	26 Feb 2025	Protocol/Protocol amendment and sponsor signatory approval
8	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	Protocol investigator agreement
9	212329 (EPI-ROTA-070 VS LAT) Protocol amendment v1.0	18 Jul 2024	ENCePP checklist for study protocols

Annex 2 Glossary of terms

Adverse event:	Any untoward medical occurrence in a study subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.
Eligible:	Qualified for enrollment into the study based upon strict adherence to inclusion/exclusion criteria.
End of study	Date of completion of data analysis.
Index date:	For the intussusception (IS) cases, the index date will be the onset date of the definite IS episode. For the controls, the hospital admission date will be considered as the index date.
Legally acceptable representative (The terms legal representative or legally authorized representative are used in some settings)	An individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the trial.
Primary completion date:	Primary completion date is defined as the date of final collection of data for all primary outcomes/endpoints.
Protocol amendment:	The International Council on Harmonization (ICH) defines a protocol amendment as: ‘A written description of a change(s) to or formal clarification of a protocol.’ GSK further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Self-contained study	Study with objectives not linked to the data of another study.

Self-controlled case series (SCCS):	Statistical method for assessing the association between a transient exposure and an adverse event. The method was developed to study adverse reactions to vaccines. The method uses only cases; no controls are required as the cases act as their own controls. Each case's given observation time is divided into control and risk periods. Risk periods are defined during or after the exposure. The method estimates a relative incidence rate, that is, the incidence in risk periods relative to the incidence in control periods. An advantage of the method is that confounding factors that do not vary with time, such as genetics, location, socio-economic status are controlled for implicitly.
Self-Controlled Risk Interval (SCRI)	The SCRI uses only vaccinated cases occurring in pre-specified risk or control intervals (“windows”). Each subject serves as its own control, thus controlling for fixed potential confounders (e.g., sex, genetic factors, socio-economic status), whether they are known or not. In addition, in using only vaccinated cases the SCRI design avoids the bias that can affect cohort studies when vaccinated subjects are misclassified as unvaccinated. The null hypothesis is that the risk of the outcome on an average day during the pre-defined risk interval after vaccination is the same as the risk of the outcome on an average day during the pre-defined control interval.
Site monitor:	An individual assigned by the sponsor and responsible for assuring proper conduct of clinical/epidemiology studies at 1 or more investigational sites.
Study population:	Sample of population of interest.
Subgroup:	A subset of the total study population sharing common characteristic (e.g., gender).
Subject number:	A unique number identifying a subject, assigned to each participant consenting to participate in the study.
Subject identifier number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the epidemiological study or a person

about whom some medical information has been recorded in a database.

Annex 3 List of Principal and Coordinating Investigators

The contact details and final list of principal and coordinating investigators will be available upon request once known.

Annex 4 Sponsor Information

1. **Sponsor:**

GlaxoSmithKline Biologicals (GSK)
GlaxoSmithKline Biologicals SA
Rue de l'Institut, 89
B-1330 Rixensart
Belgium

2. **Sponsor medical experts for the study:**

PPD Vaccine
Epidemiology
Primary Author/NI Scientific Lead

PPD
Therapy Area Leader/+1 Manager

PPD
Qualified Person for Pharmacovigilance /Delegate

Refer to the local study contact information document

3. **Sponsor study monitor**

Refer to the local study contact information document

Annex 5 Amendment and administrative changes to The protocol

Amendment or Update No	Date	Amendment or Update	Reason
1	18 Jul 2024	Updated MAH contact information	Change in MAH contact

Annex 6 Additional Information

Not applicable

Annex 7 Pro Approval

Reviewed and App

Stéphanie Barthès	PPD	26-Feb-2025
<Sponsor>		Date
Huifeng Yun	PPD	10-Mar-2025
<Sponsor>		Date
Peggy Webster	PPD	26-Feb-2025
<Sponsor>		Date

Note: Not applicable if an eSignature process is used to get the sponsor approval.

Annex 8 Protocol Amendment 1 Investigator Agreement

I agree:

- **To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the study agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline Biologicals SA (GSK).**
- **To assume responsibility for the proper conduct of the study at this site.**
- **That I am aware of and will comply with all applicable regulatory requirements.**
- **That I will comply with the terms of the site agreement.**
- **To ensure that all persons assisting me with the study are adequately informed about the marketed product and study-related duties and functions as described in the protocol.**
- **To supervise any individual or party to whom I have delegated study-related duties and functions conducted at the study-site.**
- **To ensure that any individual or party to whom I have delegated study-related duties and functions conducted at the study-site are qualified to perform those study-related duties and functions.**
- **To co-operate with a representative of GSK in the monitoring process of the study and in resolution of queries about the data.**
- **To have control of all essential documents and records generated under my responsibility before, during, and after the study.**
- **That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor and more generally about his/her financial ties with the sponsor. GSK will use and disclose the information solely for the purpose of complying with regulatory requirements.**

Hence, I:

- **Agree to supply GSK with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).**
- **Agree to promptly update this information if any relevant changes occur during the course of the study and for 1 year following completion of the study.**
- **Agree that GSK may disclose any information about such ownership interests and financial ties to regulatory authorities.**
- **Agree to provide GSK with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.**

Title	Post-marketing study to assess the risk of intussusception after immunization with GlaxoSmithKline (GSK) Biologicals' oral live-attenuated human rotavirus vaccine in infants less than 1 year old in Latin America
Date of protocol	26 Feb 2025
Investigator name	_____
Signature	_____
Date	_____

Annex 9 ENCePP Checklist for study protocols

Section 1: Milestones	Yes	No	N/A	Section Number
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[2]	☒	☐	☐	Section 6
1.1.2 End of data collection[3]	☒	☐	☐	Section 6
1.1.3 Progress report(s)	☐	☒	☐	
1.1.4 Interim report(s)	☐	☒	☐	
1.1.5 Registration in the EU PAS Register®	☐	☐	☒	
1.1.6 Final report of study results	☒	☐	☐	Section 6

Comments:

Section 2: Research question	Yes	No	N/A	Section Number
2.1 Does the formulation of the research question and objectives clearly explain:	☒	☐	☐	Section 8
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	Section 7
2.1.2 The objective(s) of the study?	☒	☐	☐	Section 8
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalized)	☒	☐	☐	Section 9.1
2.1.4 Which hypothesis(-es) is (are) to be tested?	☒	☐	☐	Section 9.7.3
2.1.5 If applicable, that there is no a priori hypothesis?	☐	☐	☒	

[2] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.

[3] Date from which the analytical dataset is completely available.

Comments:

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	Section 9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	Section 9.2.6
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☐	☐	☒	
3.4 Does the protocol specify measure(s) of association? (e.g. risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☒	☐	☐	Section 9.1.5
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☒	☐	☐	Section 11

Comments:

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	Section 9.2.1
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period	☒	☐	☐	Section 9.2.1
4.2.2 Age and sex	☒	☐	☐	Section 9.2.3
4.2.3 Country of origin	☒	☐	☐	Section 9.2.2
4.2.4 Disease/indication	☒	☐	☐	Section 9.2.5.1

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.2.5 Duration of follow-up	☒	☐	☐	Section 9.1.1
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	Section 9.2.3

Comments:

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
5.1 Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorizing exposure, measurement of dose and duration of drug exposure)	☒	☐	☐	Section 9.2.5.3
5.2 Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☒	☐	☐	Section 9.5
5.3 Is exposure categorized according to time windows?	☒	☐	☐	Section 9.2.5.3
5.4 Is intensity of exposure addressed? (e.g. dose, duration)	☒	☐	☐	Section 9.2.5.3
5.5 Is exposure categorized based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☒	☐	☐	Section 9.2.5.3
5.6 Is (are) (an) appropriate comparator(s) identified?	☒	☐	☐	Section 9.2.3 and 9.2.5.2

Comments:

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	Section 9.1.3
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	Section 9.1.3 and 9.2.6
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☒	☐	☐	Section 9.9
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilization, burden of disease or treatment, compliance, disease management)	☐	☐	☒	

Comments:

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.1 Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☐	☐	☒	
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☐	☐	☒	
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	Section 9.1.5

Comments:

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, subgroup analyses, anticipated direction of effect)	☒	☐	☐	Section 9.7.3.2 and 9.7.3.3

Comments:

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:	☒			Section 9.4
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	Section 9.3
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	Section 9.3
9.1.3 Covariates and other characteristics?	☒	☐	☐	Section 9.3
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☒	☐	☐	Section 9.3
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	Section 9.3
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	Section 9.3

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☒	☐	☐	Study Information
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☐	☒	☐	
9.3.3 Covariates and other characteristics?	☐	☒	☐	
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☐	☐	☒	

Comments:

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	Section 9.7.3
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	Section 9.5.1 and 9.5.2
10.3 Are descriptive analyses included?	☒	☐	☐	Section 9.7.3.1
10.4 Are stratified analyses included?	☐	☒	☐	
10.5 Does the plan describe methods for analytic control of confounding?	☒	☐	☐	Section 9.7.3.4
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☒	☐	
10.7 Does the plan describe methods for handling missing data?	☒	☐	☐	Section 9.7.1
10.8 Are relevant sensitivity analyses described?	☐		☒	

Comments:

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	Section 9.8
11.2 Are methods of quality assurance described?	☒	☐	☐	Section 9.8
11.3 Is there a system in place for independent review of study results?	☐	☒	☐	

Comments:

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☒	☐	☐	Section 9.1.5
12.1.2 Information bias?	☒	☐	☐	Section 9.1.5
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☒	☒	☐	Section 9.7.3.2
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☒	☐	☐	Section 9.1.6

Comments:

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/Institutional Review Board been described?	☒	☐	☐	Section 10
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	
13.3 Have data protection requirements been described?	☒	☐	☐	Section 9.8 and 10

Comments:

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Section Number**
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	Annex 5 and Annex 7

Comments:

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Section Number**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	Section 12
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	Section 12

Comments:

Note: **The Sponsor confirms his/her agreement with the completed ENCePP checklist by signing the Protocol Sponsor Signatory Approval page.**