NCT03965962

Immunogenicity and Safety of a Purified Vero Rabies Vaccine – Serum Free in Comparison with Verorab® and Imovax® Rabies, in a Simulated Rabies Post-exposure Regimen in Healthy Adults in France

Multi-center, modified double-blind (Groups 1 to 3)/open (Group 4), controlled, randomized, Phase III study in 636 subjects aged ≥ 18 years. Subjects will receive 5 vaccine injections of either VRVg-2 (Groups 1 and 4) Verorab vaccine (Group 2) or Imovax Rabies vaccine (Group 3), at day [D] 0, D3, D7, D14, and D28 (ESSEN regimen). All groups with the exception of Group 4 will additionally receive human rabies immunoglobulin (HRIG) at D0.

Statistical Analysis Plan (SAP) - Core Body Part, Amendment 1

Trial Code:	VRV13
Development Phase:	Phase III
Sponsor:	Sanofi Pasteur SA
Investigational Product(s):	Purified Vero Rabies Vaccine - Serum Free (VRVg): Purified inactivated rabies vaccine prepared on Vero cell line
Form / Route:	Liquid/Intramuscular
Indication For This Study:	Simulated treatment of rabies after potential exposure treatment
Version and Date of the SAP core body part:	Version 2.0, 26AUG2021

Version History

Previous Version(s)*	Date	Comments (optional)
1.0	20 April 2021	First version of Statistical Analysis Plan (SAP) was finalized.
1.1	25 August 2021	The SAP was amended after the first database lock and after the unblinding of the study for active phase (DBL#1, achieved on 28May2021), but prior to the final database lock (DBL#2). The SAP was updated only to correct inadvertently truncations and unnecessary statements in previous version of SAP v1.0 dated 20APR2021. Actually, no modification was made compared to what were stated in Section 12.1.1.2 and Section 12.1.2.2 in the Protocol Amendment 2, dated 27APR2020. The changes have no impact on the first statistical analyses for active phase (DBL#1) and final analysis (DBL#2). **1) Section 5.1.1.2 Statistical Methods** Fixed the statement in the last paragraph, which was not understandable, as inadvertently truncated in the former version. The following correction was made to keep consistency with protocol. The statement "*Each non-inferiority will be demonstrated if the lower limit of the 95% CI of the difference of the proportion of subjects with an RVNA titer ≥ 0.5 IU/mL at D28 to 95% will be tested only if the primary objective is demonstrated."* was updated to *"Each non-inferiority will be demonstrated if the lower limit of the 95% CI of the difference of the 2 proportions P_{VRVg-2}-$P_{control}$ is > -5% at D28.* *Overall non-inferiority will be demonstrated if both of the non-inferiority between VRVg-2 + HRIG and Verorab vaccine + HRIG and between VRVg-2+HRIG and Imovax Rabies vaccine + HRIG are demonstrated at D28."* **2) Section 5.1.2.2 Statistical Methods** Removed the paragraphs for the GM of individual titer ratio (GMTR) between two different time points on D14/D0, D28/D0 and D42/D0 within each group and

Previous Version(s)*	Date	Comments (optional)
		the 95% CI from this section. The methodology for GMT, GMTR and 95% CI was already mentioned and presented correctly in Table 5.1 and corresponding paragraph under Table 5.1 The following paragraphs were removed. "*The GM of individual titer ratio (GMTR) between two different time points on D14/D0, D28/D0 and D42/D0 within each group will be calculated, and 95% CI will be provided as follows.* *Logarithm transformation of the individual titers will be calculated. Assuming that individual log_{10}(titer) is normally distributed, the 95% CI for the difference in log_{10}(GMT) between two time points will be in the form:* $\bar{X}_1 - \bar{X}_2 \pm t(1-\alpha/2, n_1+n_2-2)\cdot S\sqrt{1/n_1+1/n_2}$ *where $\bar{X}_i = log_{10}$(GMT) is the mean of log_{10}(titer) of time point i,* *$S^2 = [(n_1-1)\,S_1^2 + (n_2-1)\,S_2^2] / (n_1+n_2-2)$ is the pooled sample variance,* *n_i and S_i^2 are the sample size and sample variance of time point i,* *$t(1-\alpha/2,\ n_1+n_2-2)$ is the 100(1-α/2) percentile of the t-distribution with degrees of freedom df = n_1+n_2-2.* *The 95% CI for the GMTR between two time points will be formed by taking the same antilogarithms of the lower and upper limits of the 95% CI for the difference in Log_{10}(GMT) between two timepoints.*"

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List of Abbreviations

ACIP	Advisory Committee on Immunization Practices
AE	adverse event
AESI	adverse event of special interest
AR	adverse reaction
CI	confidence interval
COVID-19	Coronavirus Disease 2019
CSR	clinical study report
D	day
DC	diary card
dil	dilution
eCRF	electronic case report form
FAS	full analysis set
FASI	full analysis set for immunogenicity
FDA	Food and Drug Administration
HRIG	human rabies immunoglobulin
GMT	geometric mean of titers
GMTR	geometric mean of titer ratio
IM	intramuscular
IU	international unit
LLOQ	lower limit of quantitation
MA	memory aid
MD	missing data
MedDRA	Medical Dictionary for Regulatory Activities
mL	milliliter
NA	not applicable
PEP	post-exposure
PrEP	pre-exposure
PPAS	per-protocol analysis set
PT	preferred term
Q1; Q2; Q3	first quartile; second quartile (median); third quartile
RCDC	reverse cumulative distribution curve
RFFIT	Rapid Fluorescent Focus Inhibition test
RVNA	rabies virus neutralizing antibody

SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SD	standard deviation
SOC	system organ class (primary)
TLF	table(s), listing(s), and figure(s)
V	visit
VAC	vaccination
WHO	World Health Organization

1 Trial Objectives Introduction

Currently, Sanofi Pasteur has 2 rabies vaccines registered worldwide and available on the market: Imovax® Rabies and Verorab®. Imovax Rabies, a human diploid cell vaccine (HDCV), was first licensed in 1975. It is currently registered in 19 countries, including the US, Canada, Australia and 13 European countries. On the other hand, a purified Vero rabies vaccine (PVRV), was first licensed in France in 1985 under the commercial name of Verorab, and is extensively registered worldwide in 84 countries including 10 European countries but not in the US, Canada, and Australia. Both vaccines have a well-defined safety and immunogenicity profile.

In an effort to further improve the available rabies vaccines and optimize the manufacturing process and life cycle management, Sanofi Pasteur is developing a Purified Vero rabies vaccine – serum free (SF), henceforth referred to as VRVg.

VRVg is issued from the Wistar Rabies Pitman Moore/WI 38 1503-3M strain. VRVg is highly purified with very low residual DNA content (<100 pg per dose) and it is produced without raw material derived from human or animal origin, and without antibiotics. VRVg is compliant with standards set by the European Union Pharmacopoeia, the WHO, and the US FDA.

VRVg constitutes an improvement of Verorab [redacted]

VRVg development has been conducted through stepwise adjustments based on various regulatory, pharmaceutical, and /or clinical rationales. Two formulations referred to as VRVg-1 and VRVg-2, have been subsequently explored and tested in overall, 6 clinical studies. [redacted] Sanofi Pasteur made the decision to modify the initial formulation of VRVg-1 in order to ensure an enhanced vaccine immune response. Following the results with the second formulation and the acceptance of the health authorities to go to phase III, VRVg-2, next step consists of 2 Phase III non-inferiority studies which will compare VRVg-2 with the 2 rabies vaccines marketed by Sanofi Pasteur (Verorab and Imovax Rabies vaccines) in a simulated post-exposure (PEP) Essen (5-doses) regimen in adults (the present VRV13 trial) and in a pre-exposure (PrEP) regimen comprising adult and pediatric populations (VRV12).

2 Trial Objectives

2.1 Primary Objective

Immunogenicity

To demonstrate that VRVg-2 is non-inferior to Verorab and Imovax Rabies vaccines when co-administered with HRIG, in terms of proportion of subjects achieving a rabies virus neutralizing antibody RVNA titer ≥ 0.5 international unit per mini liter (IU/mL) at D28, ie, 14 days after the fourth vaccine injection.

2.2 Secondary Objectives

Safety

To describe the safety profile of VRVg-2 versus Verorab and Imovax Rabies vaccines when co-administered with HRIG, as well as that of VRVg-2, after each vaccine injection.

Immunogenicity

To demonstrate that the proportion of subjects in the VRVg-2 + HRIG group achieving an RVNA titer ≥ 0.5 IU/mL at D28 is at least 95%.

To describe the immune response induced by VRVg-2 versus Verorab and Imovax Rabies vaccines when co-administered with HRIG, as well as that induced by VRVg-2, at D14 (7 days after the third injection), at D28 (14 days after the fourth injection) and at D42 (14 days after the fifth injection).

Description of the Overall Trial Design and Plan

Trial Design

This will be a multi-center, modified double-blind (Groups 1 to 3)/open (Group 4), controlled, randomized, Phase III study in 636 subjects aged ≥ 18 years. Subjects will receive 5 vaccine injections of either VRVg-2 (Groups 1 and 4), Verorab vaccine (Group 2) or Imovax Rabies vaccine (Group 3), at day [D] 0, D3, D7, D14, and D28 (ESSEN regimen). All groups with the exception of Group 4 will additionally receive human rabies immunoglobulin (HRIG) at D0.

Vaccination and HRIG will be administered through the intramuscular (IM) route.

Subjects will be randomized 3:1:1:1 (Group 1, 2, 3, and 4, respectively) with the resulting number of subjects assigned to each study group shown in Table 2.1.

Table 2.1: Distribution of subjects according to vaccination group

	Vaccine	Number of subjects
Group 1	VRVg-2 + HRIG	318
Group 2	Verorab® + HRIG	106
Group 3	Imovax® Rabies + HRIG	106
Group 4	VRVg-2	106

In case of any new study hold, withdrawn or out of vaccination time windows subjects might be replaced and sample size might be increased accordingly.

2.3 Trial Plan

The trial plan is summarized in Table 2.2.

Table 2.2: Study procedures

Phase III Trial, 7 visits, 1 phone call, 5 vaccinations, 4 blood samples, 7 months period per subject

Visit	V01	V02	V03	V04	V05	V06	V07	Phone Call
Visit interval	VAC1	VAC2= VAC1+3D	VAC3 = VAC1+7D	VAC4 = VAC1+14D	VAC5= VAC1+28D	VAC5+14D	VAC5+28D	VAC5*+6M
Indicative Days (D)/Months (M)	D0	D3	D7 +1D	D14 ±1D	D28 ±3D	D42 ±3D	D56 ±3D	M7 +14D
Informed consent signed	√							
Demographic data	√							
Urine pregnancy test†	√	√	√	√	√			
Physical examination‡	√	√	√	√	√	√	√	
Past and current significant medical history	√							
Inclusion & exclusion criteria	√							
Randomization	√							
Blood sampling for serology (6 mL)	√			√	√	√		
Vaccine injection	√	√	√	√	√			
HRIG administration§	√							
30-minute observation period	√	√	√	√	√			
Diary Card (DC) Memory Aid (MA) Provided Checked Collected	DC1	DC1	DC1	DC2 DC1 DC1	DC3 DC2 DC2	DC3	MA DC3 DC3	MA
Injection site reactions and Systemic Event Assessment	√	√	√	√	√	√	√	
Temporary contraindications		√	√	√	√			
Definitive contraindications		√	√	√	√			
Reportable concomitant medication	√	√	√	√	√	√	√	
Termination Record							√	
Pregnancy cases	Collected throughout the entire study period							
SAEs and AESIs	Collected throughout the entire study period							

* VAC5 or last vaccine injection in the event of early terminated subject, contacted for the 6 months follow-up period through a phone call

†For subjects of childbearing potential

‡ Complete physical examination at V01 and medically-driven physical examination for remaining visits

§With the exception of subjects from Group 4 (who will receive VRVg-2 only)

Blood sampling

All subjects will provide blood samples before the first vaccination at D0 (baseline titer) and for the assessment of the immune response to the rabies vaccines at D14 (7 days after the third vaccine injection), D28 (14 days after the fourth vaccine injection), and D42 (14 days after the fifth vaccine injection).

Collection of safety data

Safety will be assessed in all subjects during the vaccination period and up to 28 days after vaccinations, in terms of occurrence of adverse events (AEs), serious adverse events (SAEs) and adverse events of special interest (AESIs). In addition, SAEs and AESIs will be collected up to 6 months after the last vaccination in all subjects.

3 Endpoints and Assessment Methods

3.1 Primary Endpoints and Assessment Methods

See Section 9.1 of the protocol.

3.2 Secondary Endpoints and Assessment Methods

See Section 9.2 of the protocol.

3.3 Derived Endpoints: Calculation Methods

3.3.1 Safety

Terms used in the standard safety tables to describe the safety events are specified below.

- AE: Adverse event includes solicited reaction and unsolicited event (including non-serious and serious adverse events).
- Adverse Reaction (AR): Corresponds to AE related to the study vaccine (investigational product), unless otherwise specified.
- Immediate: Unsolicited systemic AE checked “Yes” in the field of “immediate (within 30 minutes from the vaccination)” by the Investigator in the electronic case report form (eCRF).
- Solicited reaction: Event pre-listed in the eCRF, and which occurred during the solicited period (up to 7 days after the day of each vaccination).
- Unsolicited AE: AE recorded in the eCRF as unsolicited systemic event or unsolicited injection site reaction.
- Unsolicited injection site reactions are to be considered as related to the study vaccine injection and therefore will be analyzed as ARs.

Note: HRIG being an additional study medication (non-investigational product), any AEs occurring at HRIG injection location will be reported as unsolicited systemic events in this study.

- Unsolicited AEs will be analyzed up to 28 days after any and each vaccination. Unsolicited AEs occurring before or after the defined period will not be presented in the summary for unsolicited AEs but will be presented in a separate listing. The exceptions are SAEs and AESIs which are unsolicited AEs collected during the whole study period (after the first vaccination to end of 6 months safety follow-up period).
- SAE: Unsolicited AE considered serious by the Investigator (reconciled with Global Pharmacovigilance database).
- AESI: Is one of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor can be appropriate. The Medical Dictionary for Regulatory Activities (MedDRA) preferred terms (PT) defined in the protocol are:
  - Anaphylactic reaction (PT)
  - Encephalitis (PT)
  - Convulsion (PT)

3.3.1.1 Solicited Reactions

3.3.1.1.1 Daily Intensity

All daily records for solicited reactions will be derived into daily intensity according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing (Unknown).

For the derivation of daily intensity, the following sequential steps will be applied:

1) For solicited reactions (except for fever) with an Investigator presence recorded as "No" in eCRF and with all daily records missing (unknown), and then all daily intensities will be derived as "None."
2) For a temperature partially missing after decimal point, the data will be analyzed replacing "MD" (missing data) by zero. For example, a "39.MD" daily temperature will be considered as "39.0°C" at the time of analysis.
3) For non-measurable solicited reactions, daily intensities will correspond to daily records reported in the clinical database. For measurable solicited reactions, the daily measurements reported in the clinical database will be converted based upon the intensity scales defined in the protocol; this assumes a reaction that is too large to measure (not measurable [NM]) is Grade 3. Note the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the Investigator (eg, swelling measurement > 0 mm but < 25 mm).

Note: The maximum intensity on the ongoing period is derived from the record of the maximum intensity/measurement after the end of the solicited period following the rule described above.

3.3.1.1.2 Maximum Overall Intensity

Maximum overall intensity is derived from the daily intensities computed as described in Section 3.3.1.1.1 and is calculated as the maximum of the daily intensities over the period considered.

3.3.1.1.3 Presence

Presence is derived from the maximum intensity on the period considered:

- None: No presence
- Grade 1, Grade 2, or Grade 3: Presence
- Missing (Unknown): Missing presence

Subjects with at least one non-missing presence for a specific endpoint will be included in the analysis. Conversely, those without a non-missing presence will not be included in the analysis of the endpoint.

3.3.1.1.4 Time of Onset

Time of onset is derived from the daily intensities computed as described in Section 3.3.1.1.1. It corresponds to the first day with intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (ie, reaction occurs over two separate periods of time intervened by at least one daily intensity Missing or None) then the time of onset is the first day of the first occurrence.

Note: for solicited systemic reactions, if a reaction is ongoing at the time of the next vaccination, it will be treated differently depending on whether or not it has increased in intensity following the later vaccination.

- If it has not increased in intensity after the next vaccination, it is to be attributed to the earlier vaccination, and is counted as just a single occurrence in safety analysis. The time of onset is the first day of the first occurrence in the earlier vaccination.
- If it has increased in intensity after the next vaccination, it will be counted as 2 separate occurrences after each vaccination in safety analysis (the 2 occurrences will be attributed to the earlier vaccination and later vaccination, respectively). The date of the later vaccination will be considered to be the end date for the first occurrence and the start date of the second occurrence, respectively.

3.3.1.1.5 Number of Days of Occurrence During the Solicited Period

Number of days of occurrence over the solicited period considered is derived from the daily intensities computed as described in Section 3.3.1.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. Number of days of occurrence on the solicited period with a specified intensity may also be derived.

3.3.1.1.6 Overall Number of Days of Occurrence

If a reaction is ongoing at the end of the solicited period, then the overall number of days of presence is derived from the daily intensities and the stop date of the reaction after the end of the solicited period. The overall number of days of presence is:

- (stop date – last vaccination date) + (number of days of presence within the solicited period) – length of the solicited period + 1

If the stop date is missing or incomplete (contains missing data [MD]), the overall number of days of occurrence will be considered as "Missing".

3.3.1.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed as described in Section 3.3.1.1.1 and the maximum intensity on the ongoing period. The investigator's ongoing flag is not used because the measurement would determine the ongoing status of the reaction.

- Ongoing: if the last daily intensity of the solicited period is at least Grade 1 and the maximum intensity in the ongoing period is at least Grade 1
- Not ongoing: if the last daily intensity of the solicited period is None or the maximum intensity on the ongoing period is None.
- Missing: all other conditions (in this case, it is not included in the denominator of the ongoing analysis in the safety tables).

3.3.1.2 Unsolicited AEs (Including SAE and AESI)

3.3.1.2.1 Presence

An observation will be considered an event if it has at least a verbatim term and is not a Grade 0 intensity event.

Grade 0 events should not be included in safety analysis but are included in the separate listing "Unsolicited adverse events not included in the safety analysis."

3.3.1.2.2 Intensity

Intensity for unsolicited AEs will be derived according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

If the unsolicited AE is measurable and its PT is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule as the intensity scales defined in the protocol for that measurable injection site or systemic reaction. Note the intensity could be considered as "None" (not a reaction) in the analysis despite being considered a reaction by the Investigator (eg, swelling measurement > 0 mm but < 25 mm).

Intensity for the other unsolicited AEs will correspond to the value reported in the eCRF.

The maximum intensity corresponds to the highest intensity for a unique term.

3.3.1.2.3 Last Vaccination

Last vaccination before an unsolicited injection site AE is derived from the vaccination number in the "Vaccination" field provided in the eCRF.

Last vaccination before an unsolicited systemic AE is derived from the start date of the systemic unsolicited AE provided in the eCRF and is calculated as follows:

- If an unsolicited systemic AE has a complete start date and different to any of the vaccination dates, the start date is used to determine the last vaccination before the unsolicited AE
- If the start date is missing or partially missing, or equal to any vaccination date, then the visit number in the "Appeared after Visit" field, is used to determine the last vaccination before the unsolicited systemic AE

3.3.1.2.4 Time of onset

Time of onset is derived from the start date of the unsolicited AE and the date of last vaccination as described in Section 3.3.1.2.3:

- Time of onset = start date of the unsolicited AE – date of last vaccination before the unsolicited AE

The time of onset should be considered as missing only if one or both dates are missing or partially missing.

An unsolicited AE with missing time of onset will be considered to have occurred just after the last vaccination (computed according to Section 3.3.1.2.3), so will be included in safety analysis. Note: Unsolicited AE that occurred before the first vaccination (negative time of onset) will not be included in safety analysis but will be listed separately.

3.3.1.2.5 Duration

Duration is derived from the start and stop dates of the unsolicited AE:

- Duration = stop date of unsolicited AE - start date of unsolicited AE + 1.

The duration should be considered as missing only if one or both of the start and stop dates of the unsolicited AE is missing or partially missing.

3.3.1.2.6 Serious Adverse Events

An unsolicited event will be considered as a SAE if "Yes" is checked for "Serious" in the CRF.

3.3.1.2.7 Adverse Events of Special Interest

An unsolicited event will be considered as an AESI if "Yes' is checked for "Is the event an AESI?" in the CRF.

3.3.1.3 Other Safety Endpoints

3.3.1.3.1 Pregnancy

This information will not be included in the analysis but will be listed separately. No derivation or imputation will be done.

3.3.1.3.2 Action Taken

This information will be summarized as collected, including missing observations. No derivation or imputation will be done.

3.3.1.3.3 Seriousness

This information will be summarized as collected. No derivation or imputation will be done.

3.3.1.3.4 Outcome

This information will be summarized as collected. No derivation or imputation will be done.

3.3.1.3.5 Causal Relationship

This information will be summarized as collected in the field "Relationship to investigational product". Missing causal relationship to study vaccine (investigational product) will be handled as described in Section 4.3.2.2.

Relationship to study procedures for SAE is only presented in the listing.

3.3.1.3.6 Adverse Events Leading to Study Discontinuation

Adverse Events Leading to Study Discontinuation are defined as AEs leading to discontinuation of the study during active phase (from Visit 1 up to Visit 7). AEs leading to discontinuation of the study during safety follow-up phase (between Visit 7 and Phone call at Month 7) are not to be considered for this category. This information will be summarized as collected. A flag is available in the clinical database for all AEs in order to identify AEs leading to study discontinuation before the end of active phase.

In general, the items that are counted are:

- Disposition table: A subject who, on the "Completion at End of Study" form question "What was the participant's status?" has "Adverse Event" checked.
- Safety overview table: A subject who has either on the "Completion at End of Study" form, question" What was the participant's status?" has "Adverse Event" checked or lists a solicited AE that has "Caused Study Termination" checked that is at least Grade 1 or an unsolicited AE that has "Caused Study Discontinuation" checked that is at least Grade 1 or missing and is within the time period indicated.
- System Organ Class (SOC)/Preferred Term (PT) table: A solicited AE that has "Caused Study Termination" checked that is at least Grade 1 or an unsolicited AE that has "Caused Study Discontinuation" checked that is at least Grade 1 or missing and is within the time period indicated.

3.3.2 Immunogenicity

3.3.2.1 Computed Values for Analysis

In order to appropriately manage duplicate values for analysis purposes, the individual geometric mean (GM) of both values will be computed for each blood sample.

If a reported value is < Lower Limit of Quantitation (LLOQ) (ie, 0.2 IU/mL), then the computed value LLOQ/2 (ie, 0.1 IU/mL) will be used for the GM calculation.

For the qualitative Advisory Committee on Immunization Practices (ACIP) criteria reading (stated as Complete or Incomplete twice for each sample), the individual ACIP status will be derived as follows:

- If the 2 values are Complete, then the sample status will be Complete
- If the 2 values are Incomplete, then the sample status will be Incomplete
- If 1 value is Complete and the other one is Incomplete, the status will be Undefined
- If 1 value is Complete and other one is Not Reported, the status will be Complete
- If 1 value is Incomplete and other one is Not Reported, the status will be Incomplete
- If 2 values are Not Reported, the status will be Not Reported

There will be no Upper Limit of Quantitation (ULOQ) for the Rapid Fluorescent Focus Inhibition test (RFFIT) assay.

3.3.3 Efficacy

Not applicable.

3.3.4 Derived Other Variables

3.3.4.1 Duration of a Subject in the Study

The duration of a subject in the whole study (from Visit 1 up to end of 6-month follow up) is computed as: Maximum (Visit dates, Active phase termination date, Follow-up date) – Visit 01 date +1.

3.3.4.2 Duration of the Study

The duration of the active phase (from Visit 1 up to Visit 7) is computed as follows: Maximum of all subjects (Visit dates, Active phase termination date) - Minimum of all subjects (Visit 01 date) +1.

The duration of the follow-up is computed as: Maximum of all subjects (Follow-up date) - Minimum of all subjects (Follow-up date) + 1

The duration of the whole study (from Visit 1 up to end of 6-month follow up) is computed as: Maximum of all subjects (Visit dates, Active phase termination date, Follow-up date) – Minimum of all subjects (date of V01) +1.

3.3.4.3 Age and Age group

Age group is classified based on the age in years collected in eCRF, and the following age group will be derived in subgroup analysis: ≥18 to 40 years, ≥41 to 64 years, and ≥65 years.

The quantitative descriptive statistics (eg, Mean, SD, Max, Min, Median, Q1 and Q3) of age in demographics summary table(s) is based on the age in year collected in eCRF as well.

3.3.4.4 Vaccination Dose

For the same type of vaccine, if one vaccination was divided into several injections at one visit, all injections given at different sites within the same visit were considered as a single dose

4 Statistical Methods and Determination of Sample Size

The statistical analysis will be performed under the responsibility of the Sponsor's Biostatistics platform with the SAS software, at least version 9.4 (SAS Institute, Cary, North Carolina, USA).

The statistical analysis will be performed in 2 steps. A first statistical analysis will be carried out once all the safety and immunogenicity data collected up to D56, ie, up to 28 days after the last vaccine injection, will be obtained and locked. Then an addendum will be performed after the 6-month safety follow-up.

The results of the statistical analysis will be available in the final clinical study report (CSR).

For descriptive purposes, the following statistics will be presented:

Table 4.1: Descriptive statistics produced

Baseline characteristics and follow-up description	**Categorical data**	Number of subjects. Percentage of subjects.
	Continuous data	Mean, standard deviation, median, quartiles, minimum, and maximum.
Clinical safety results	**Categorical data**	Solicited: Number and percentage (95% confidence interval [CI) of subjects. Unsolicited: Number and percentage (95% CI) of subjects, and number of events.
Immunogenicity results	**Categorical data (cutoff)**	Number and percentage (95% CI) of subjects.
	Continuous data (titer / data)	Log10: Mean and standard deviation. Anti-Log10 (work on Log10 distribution, and anti-Log10 applied): Geometric mean, 95% CI of the geometric mean, median, quartiles, minimum, and maximum. Graphical representation by Reverse Cumulative Distribution Curve (RCDC).

The CI for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (1), ie, using the inverse of the beta integral with SAS®).

For immunogenicity results, assuming that Log10 transformation of the titers / data follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log_{10} (titers / data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide GMs and their 95% CI.

GM is defined as follows:

$$GM = \left(\prod_{i=1}^{n} y_i \right)^{1/n} = 10^{\wedge} \left(\frac{1}{n} \sum_{i=1}^{n} \log_{10}(y_i) \right),$$

where $(y_1, y_2, \ldots, y_n)$ are the observed titers or individual ratios for each subject.

4.1 Statistical Methods

4.1.1 Hypotheses and Statistical Methods for Primary Objective

4.1.1.1 Hypotheses

The immunogenicity of VRVg-2 + HRIG will be compared to that of both Verorab + HRIG and Imovax Rabies vaccine + HRIG at D28, i.e., 14 days after the fourth vaccine injection, using a non-inferiority testing.

For each comparison, the primary parameter will be the difference of the proportion of subjects with an RVNA titer ≥ 0.5 IU/mL at D28 between the 2 compared vaccine groups. The hypotheses tested will be the following:

H_0: $P_{VRVg\text{-}2+HRIG}$-$P_{control} \leq$ -5%

H_1: $P_{VRVg\text{-}2+HRIG}$-$P_{control}$ > -5%

With P= proportion of subjects with an RVNA titer ≥ 0.5 IU/mL at D28 (%).

VRVg-2 will be considered as non-inferior to the tested control if the hypothesis H0 is rejected.

4.1.1.2 Statistical Methods

For the non-inferiority hypothesis testing, the statistical methodology will be based on the use of the two-sided 95% CI of the difference of proportions of subjects with an RVNA titer ≥ 0.5 IU/mL at D28 between the VRVg-2 vaccine + HRIG group (Group 1) and the comparator vaccine groups (Group 2 and Group 3). The 95% CI for differences will be calculated using Wilson score method without continuity correction (2).

Let $\hat{\theta} = p_1 - p_2$, then $L = \hat{\theta} - \delta$ and $U = \hat{\theta} + \varepsilon$ are respectively the lower and the upper limits of the CI, where:

$$\delta = Z_{0\,025}\sqrt{\left\{\frac{l_1(1-l_1)}{n_1} + \frac{u_2(1-u_2)}{n_2}\right\}}$$

$$\varepsilon = Z_{0\,025}\sqrt{\left\{\frac{l_2(1-l_2)}{n_2} + \frac{u_1(1-u_1)}{n_1}\right\}}$$

l_1 and u_1 are calculated from the CI of the single proportion in Group 1 given by:

$$\frac{(2n_1p_1 + Z^2_{0\,025} \pm Z_{0\,025}\sqrt{(Z^2_{0\,025} + 4n_1p_1(1-p_1))})}{2(n_1 + Z^2_{0\,025})}$$

l_2 and u_2 are calculated from the CI of the single proportion in comparator vaccine group (Group 2 or Group 3) given by:

$$\frac{(2n_2p_2 + Z^2_{0\,025} \pm Z_{0\,025}\sqrt{(Z^2_{0\,025} + 4n_2p_2(1-p_2))}}{2(n_2 + Z^2_{0\,025})}$$

where $Z_{0.025}$ is the upper 97.5th percentile of the standard normal distribution.

Each non-inferiority will be demonstrated if the lower limit of the 95% CI of the difference of the 2 proportions $P_{VRVg\text{-}2}$-$P_{control}$ is > -5% at D28.

Overall non-inferiority will be demonstrated if both of the non-inferiority between VRVg-2 + HRIG and Verorab vaccine + HRIG and between VRVg-2+HRIG and Imovax Rabies vaccine + HRIG are demonstrated at D28.

4.1.2 Hypotheses and Statistical Methods for Secondary Objectives

The superiority of the proportion of subjects with an RVNA titer ≥ 0.5 IU/mL at D28 to 95% will be tested only if the primary objective is demonstrated.

4.1.2.1 Hypotheses

If the primary objective is reached, the following hypotheses will be tested at D28, as part of the secondary objectives:

H0: $P_{VRVg\text{-}2+HRIG} < 95\%$

H1: $P_{VRVg\text{-}2+HRIG} \geq 95\%$

The proportion of subjects with an RVNA titer ≥ 0.5 IU/mL at D28 after receiving VRVg-2 will be considered as equal to or higher than 95% if the hypothesis H0 is rejected.

4.1.2.2 Statistical Methods

The superiority testing will be demonstrated if the lower limit of the 95% CI of the proportion, calculated using the exact binomial distribution (1), is equal to or higher than 95%.

All other secondary endpoints will be described by vaccine group using descriptive statistical methods, as suggested in Table 5.1.

Immunogenicity endpoints

For all the subjects, a descriptive analysis for pre-Dose 1 (D0), 7 days post-Dose 3 (D14), 14 days post-Dose 4 (D28), and 14 days post-Dose 5 (D42) will present the following statistics for RVNA titers in each vaccine group:

- Number of subjects with RVNA titer available
- Number and percentage of subjects with RVNA titer ≥ 0.2IU/mL (LLOQ) and 95% CI of the proportion
- Number and percentage of subjects with RVNA titer ≥ 0.5 IU/mL, and 95% CI of the proportion
- Number and percentage of subjects with complete or incomplete results at the starting dilution (1/5) of the RFFIT assay

- Among the analysis population
- Among the subset of subjects with a determined result (identical duplicate values, either Complete or Incomplete)

- GMTs and 95% CI of RVNA titers
- GM of individual titer ratio (GMTR) between the post vaccination time points (D14, D28, and D42) and the baseline (D0) within each group, and the 95% CIs:
  - D14/D0
  - D28/D0
  - D42/D0
- Distribution of titers (minimum, Q1, median, Q3, maximum): based on Log10 transformation first and then antilog transformation.
- Log_{10} (Titer): mean and its standard deviation (SD)
- Reverse Cumulative Distribution Curves (RCDC) of RVNA titers at D0, D14, D28, and D42.

The complete descriptive immunogenicity data will be analyzed on the per-protocol analysis set (PPAS) primarily.

Safety endpoints

The analysis will be descriptive. The statistics presented in Table 4.1 will be produced, based on the Safety Analysis Set (SafAS).

The safety analysis will report the occurrence of solicited reactions and the incidence of unsolicited AEs including SAEs and AESIs, over the safety observation period according to the vaccine received.

- Immediate unsolicited systemic AEs reported in the 30 minutes after each and vaccination.
- Solicited injection site reactions occurring within 7 days after each injection.
- Unsolicited injection site reactions occurring within 28 days after each injection.
- Solicited systemic reactions occurring between the first and the second injection, between the second and the third injection, and up to 7 days after the remaining injections.
- Unsolicited systemic AEs occurring between each injection and up to 28 days after the last injection.
- SAEs throughout the trial, ie, up to 6 months after the last injection.
- AESIs throughout the trial, ie, up to 6 months after the last injection.

In order to avoid any under-estimation of the incidences, the number of subjects with documented safety will be used as denominator of the frequencies.

- For solicited reactions, the denominator is the total number of subjects who have non-missing data for the particular category of reaction during the time period concerned.

  Note: for solicited systemic reactions, if a reaction is ongoing at the time of the next vaccination, it will be treated differently depending on whether or not it has increased in intensity following the later vaccination.

  - If it has not increased in intensity after the next vaccination, it is to be attributed to the earlier vaccination, and is counted as just a single occurrence in safety analysis. The time of onset is the first day of the first occurrence in the earlier vaccination.
  - If it has increased in intensity after the next vaccination, it will be counted as 2 separate occurrences after each vaccination in safety analysis (the 2 occurrences will be attributed to the earlier vaccination and later vaccination, respectively). The date of the later vaccination will be considered as the end date for the first occurrence and the start date of the second occurrence, respectively.

- For unsolicited events, the denominator is the total number of subjects who were vaccinated at the dose analyzed (for the analyses after each dose) or the total number of subjects who were vaccinated at least one dose (for the analyses after any dose).

For safety parameters, 95% CIs of point estimates of proportion will be calculated using the exact binomial distribution (Clopper-Pearson method) for proportions (1).

30-minutes Post-Vaccination Observation Period

Unsolicited systemic AEs occurring within 30 minutes after each vaccination will be presented in summary safety tables, analyzed according to their causality (relationship to the study vaccine) and Grade 3 intensity.

Solicited Reactions

The solicited injection site reactions and the solicited systemic reactions will be presented according to the term listed in the eCRF, separately.

The solicited injection site reactions will be analyzed within 7 days after each vaccination (from D0 to D7), and within 7 days after any vaccination.

The solicited systemic reactions will be analyzed between each vaccination if vaccinations are separated less than 7 days, and up to 7 days after each vaccination if vaccinations are separated by 7 days or more.

Each type of solicited reactions will be presented after each injection according to:

- Maximum intensity during the solicited period and overall for on-going solicited reactions:

- Grade 1
- Grade 2
- Grade 3

- Time of onset categories are in Table 4.2

Table 4.2: Time of onset categories for solicited reactions

	Injection Site Reactions	Systemic Reactions
Post-Dose 1	D0-D3 D4-D7	D0-D3
Post-Dose 2		D0-D3 D4
Post-Dose 3, 4, 5		D0-D3 D4-D7

- *Range of number of days of occurrence categories are in* Table 4.3

Table 4.3: Range of number of days of occurrence categories for solicited reactions during the solicited period

	Injection Site Reactions	Systemic Reactions
Post-Dose 1	1 - 3 days 4 - 7 days 8 days	1 - 3 days 4 days
Post-Dose 2		1 - 3 days 4 - 5 days
Post-Dose 3, 4, 5		1 - 3 days 4 - 7 days 8 days

- *Range of overall number of days of occurrence categories are in* Table 4.4

Table 4.4: Range of overall number of days of occurrence categories for solicited reactions during the solicited period

	Injection Site Reactions	Systemic Reactions
Post-Dose 1	1 - 3 days 4 - 7 days 8 days or more Missing end date	1 - 3 days 4 days 5 days or more Missing end date

Post-Dose 2		1 - 3 days 4 - 5 days 6 days or more Missing end date
Post-Dose 3, 4, 5		1 - 3 days 4 - 7 days 8 days or more Missing end date

- *Action Taken*

- Missing
- None
- Medication
- Healthcare provider contact
- Hospitalization
- Discontinuation of the study vaccination

Solicited reaction after any injection

"After any injection" means after all scheduled vaccine injections, and at least after the first vaccine injection (D0).

The solicited reaction will be presented after any injections according to:

- *Number of days of occurrence over the D0-D7 period for injection site reactions or between vaccinations for systemic reactions (up to D7 for the last 3 vaccinations)*

- *Time of onset period*

- *Intensity grade: the maximum intensity grade will be computed during the solicited period for all solicited reactions and overall for ongoing solicited reactions.*

Unsolicited Adverse Events

The unsolicited systemic AEs will be analyzed:

- "Between the first and the second vaccine injection", which corresponds to AEs reported at V02 and occurring after the first vaccine injection-[a]

[a] For subjects interrupting their vaccination schedule after the first injection, unsolicited AEs until 28 days after the first injection will be collected

- "Between the second and the third vaccine injection", which corresponds to AEs reported at V03 and occurring after the second vaccine injection [a]

- "Between the third and the fourth vaccine injection", which corresponds to AEs reported at V04 and occurring after the third vaccine injection [b]

- "Between the fourth and the fifth vaccine injection", which corresponds to AEs reported at V05 and occurring after the fourth vaccine injection [c]

- "Within 28 days after the fifth vaccine injection", which corresponds to AEs reported at V07 and occurring after the fifth vaccine injection

- "Within 28 days after any vaccine injection", which corresponds to AEs belonging to any of the five above categories

The unsolicited injection site reactions will be analyzed within 28 days (from D0 to D28) after each vaccination, and within 28 days after any vaccination. An unsolicited injection site reaction (**excluding SAE and AESI**) reported with an onset > 28 days after a vaccination will not be included in the safety analysis, but will be listed separately.

The unsolicited systemic AEs will be analyzed between each vaccination if vaccinations are separated less than 28 days, or up to 28 days after each vaccination, if vaccinations are separated by 28 days or more or the next vaccination is interrupted, and up to 28 days after any vaccination. An unsolicited systemic AE (**excluding SAE and AESI**) reported with an onset > 28 days after vaccination will not be included in the safety analysis, but will be listed separately.

Note: An unsolicited AE with missing time of onset will be considered to have **occurred within or up to 28 days after the last vaccination** (computed according to Section 3.3.1.2.3), so will be included in safety analysis.

The unsolicited AEs will be summarized in the safety overview and analyzed according to their nature (SOC and PT of the MedDRA classification), causality (relationship to the study vaccine), time of onset, duration and maximum intensity.

The occurrence of any unsolicited AE will be presented after any and each injection according to:

- *Maximum intensity:*

  - Missing
  - Grade 1
  - Grade 2
  - Grade 3

[a] For subjects interrupting their vaccination schedule after the second injection, unsolicited AEs until 28 days after the second injection will be collected

[b] For subjects interrupting their vaccination schedule after the third injection, unsolicited AEs until 28 days after the third injection will be collected

[c] For subjects interrupting their vaccination schedule after the fourth injection, unsolicited AEs until 28 days after the fourth injection will be collected

- Time of onset categories:

- Missing
- D0-D3
- D4-D7
- D8-D14
- ≥D15

- Maximum duration:

- Missing
- 1 - 3 days
- 4 - 7 days
- 8 - 14 days
- 15 days or more

SAEs and AESIs

In addition, the SAEs and AESIs, which are included in the unsolicited AEs, occurred during the study will also be analyzed on the following periods:

1. during vaccination period: from date of first vaccination (D0) to 28 days after the date of last exposure to study vaccine (date of last exposure + 28 days)
2. during the six-month safety follow-up period: from 29 days after the date of last exposure to study vaccine (date of last exposure + 29 days) to end of study (last visit date or contact date collected in eCRF)
3. during the study: from date of first vaccination (D0) to end of study [last visit date or contact date collected in eCRF])

SAEs will be analyzed according to their causality (relationship to the study vaccine), seriousness criteria, outcome and nature (SOC and PT of the MedDRA classification).

AESIs will be summarized and analyzed according to their nature (SOC and PT of the MedDRA classification) and causality.

4.1.3 Exploratory Analyses

4.1.3.1 Subgroup Analysis: Impact of Demographic Factors and Center

With the aim to provide the same standards and granularity in terms of investigational results across VRVg studies, and comply with health authorities requirements (3), the possible influence of several covariates on the safety and immunogenicity results will be studied using descriptive statistics. Thus, the main immunogenicity and safety parameters will be described according to gender, age group, ethnicity (only if more than 5% of subjects had different ethnicity), race (only if more than 5% of subjects had different race), and center.

Race and ethnicity will be defined according to current guidelines (3).

Age groups are the following ones: ≥ 18 to 40 years, ≥ 41 to 64 years, and ≥ 65 years.

These exploratory analyses will be performed on the FASI (for immunogenicity) or SafAS population (for safety).

In case of any safety signal detected in the VRVg-2 vaccines, a statistical comparison will be performed between VRVg-2 groups (VRVg-2+HRIG and/or VRVg-2) and each of the control groups with a Fisher exact test in order to check for statistical significance (alpha=0.05, two sided).

4.1.3.2 Impact of the COVID-19 pandemic

To evaluate the possible impact of the COVID-19 pandemic on the study conduction, main immunogenicity and safety results, the following sensitive analyses will be performed but not limited to:

- Impact of COVID-19 pandemic on subject's disposition, based on enrolled subjects

  Note: Subjects impacted by COVID-19 pandemic situation correspond to subjects with at least one major/critical protocol deviation due to COVID-19 or who did not complete the study due to COVID-19 or who reported an impact on 6-month follow up in pandemic form
- Impact of COVID-19 pandemic on immunogenicity analysis, based on FASI.
- Impact of medical history of suspected/confirmed COVID-19 pandemic before enrollment on immunogenicity analysis, based on FASI.
- Impact of the use of concomitant medications that are used during the study/ or were used not over 2 months before enrollment for treating/preventing COVID-19 (ie, Hydroxychloroquine and chloroquine) on immunogenicity based on FASI.
- Immunogenicity of subjects who did not take any concomitant medications for treating/preventing COVID-19 (ie, Hydroxychloroquine and chloroquine) within 2 months before enrollment based on PPAS
- Impact of COVID-19 pandemic on safety overview up to 28 days after any vaccination, based on SafAS.
- Impact of medical history of suspected/confirmed COVID-19 before enrollment on safety overview up to 28 days after any vaccination, based on SafAS.
- Impact of the use of concomitant medications that are used during the study/ or were used at least 2 months before enrollment for treating/preventing COVID-19 (ie, Hydroxychloroquine and chloroquine) on safety overview up to 28 days after any vaccination, based on SafAS.

Note: If only few subjects were impacted by COVID-19 (<5% in randomized subjects), only the corresponding listings will be provided instead of tables for immunogenicity and safety evaluation. Exception is the immunogenicity evaluation for the subjects who did not take any concomitant

medications for treating/preventing COVID-19 (ie, Hydroxychloroquine and chloroquine) within 2 months before enrollment base on PPAS.

4.1.3.3 Safety overview analysis for subjects who had 5 doses

Because of study hold due to SAE and COVID-19, a proportion of subjects didn't complete the full vaccination schedule. To evaluate the safety events for subjects who had 5 doses, summary of safety overview will be performed on subjects who had 5 doses without consider time window.

4.2 Analysis Set

4.2.1 Full Analysis Set

The Full Analysis Set (FAS) is defined as the subset of randomized subjects who received at least 1 dose of the study vaccines.

The FASI is defined as a subset of the FAS, defined as all subjects from FAS who have a baseline RVNA titer lower than 0.5 IU/mL.

The analysis of immunogenicity addresses endpoints involving pre- and post-injection titers. The analysis will include all available data for each time point.

4.2.2 Per-Protocol Analysis Set

The PPAS is a subset of the FAS.

As D28 is the timepoint used for primary objective assessment, the PPAS will be defined at D28 (V05). The subjects presenting with at least one of the following relevant protocol deviations before D28 (ie, 14 days after the fourth vaccine injection) will be excluded from the PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not receive the first 4 doses of the vaccination schedule
- Subject received a vaccine other than the one that he / she was randomized to receive
- Preparation and / or administration of vaccine and concomitant medication (including HRIG for groups 1 to 3) was not done as per-protocol for the first 4 vaccinations
- Subject did not receive the first 4 vaccine injections in the proper time window
    - Vaccination 2 in [D03-D05]
    - Vaccination 3 in [D07-D09]
    - Vaccination 4 in [D13-D16]
- Subject did not provide the post-Dose 4 serology sample at V05 in the proper time window [D25-D31] from D0

- Subject received a protocol-prohibited therapy/medication/vaccine (belonging to categories 2 and 3) before V05
- Subject's serology sample is missing or did not produce valid test results at D0 or D28
- Seropositive subject at D0, ie, RVNA titer ⩾ LLOQ
- Subject developed a protocol-specified withdrawal criterion during the study but was not withdrawn
- Subject did not receive the correct amount of HRIG at D0

Adherence to the definition of the PPAS analysis set may also be decided during the blinded data review, ie, before breaking the code and locking the database.

4.2.3 Safety Analysis Set

The SafAS is defined for each dose as the subset of subjects having received this dose. All subjects will have their safety analyzed after each dose according to the study vaccine they actually received and after any dose according to the study vaccines received at the first dose.

Safety data recorded for a vaccine received out of the protocol design will be excluded from the analysis (and listed separately). Thus, if a subject does not receive any study vaccine at a given visit or if the study vaccine received does not correspond to any protocol group at a specific dose, the subject is excluded from the SafAS at this dose; however, the subject will be included in the analysis for all doses combined (referred to as analysis "after any dose") according to the first dose received that corresponds to a protocol group.

Note:

- If one subject received both VRVg-2 vaccine and HRIG at first dose, the vaccination group for safety analysis is Group 1;
- If one subject received Verorab vaccine or Imovax Rabies vaccine at first dose, no matter HRIG was concomitantly administered or not, the vaccination group for safety analysis is Group 2 or Group 3;
- If one subject received VRVg-2 vaccine only at first dose, the vaccination group for safety analysis is Group 4;
- If one subject received HRIG only at first dose, the vaccination group for safety analysis is "Other" and will be excluded from safety analysis.

4.2.4 Other Analysis Set(s)

Enrolled subjects
Enrolled subjects are subjects for whom an eCRF has been created.
Randomized subjects
A randomized subject is a subject for whom a vaccine group has been allocated.

4.2.5 Populations Used in Analyses

The following table presents populations used in the statistical analysis.

Table 4.5: Populations used in the analyses

		Analysis sets	Analysis by
Primary Objective	**D28 non-inferiority testing**	PPAS (main analysis), FAS/FASI	Received vaccine group Randomized vaccine group
Secondary Objectives	**D28 Superiority testing**	PPAS (main analysis), FAS/FASI	Received vaccine group Randomized vaccine group
	Immunogenicity description	PPAS (main analysis), FAS/FASI	Received vaccine group Randomized vaccine group
	Safety description	SafAS	Received vaccine group
Exploratory analyses	**Main immunogenicity endpoints**	FASI (main analysis) PPAS*	Randomized vaccine group Received vaccine group
	Main safety endpoints	SafAS	Received vaccine group

*Note: Immunogenicity of subjects who did not take any concomitant medications for treating/preventing COVID-19 (ie, Hydroxychloroquine and chloroquine) within 2 months before enrollment

4.3 Handling of Missing Data and Outliers

4.3.1 Immunogenicity

No imputation of missing values and no search for outliers will be performed.

4.3.2 Safety

4.3.2.1 Immediate

For unsolicited systemic AEs, a missing response to the “Immediate” field is assumed to have occurred after the 30-minute surveillance period and will not be imputed.

4.3.2.2 Causality

By convention, all events reported at the study vaccine injection site (either solicited or unsolicited) will be considered as related to the study vaccine (investigational medicinal product) and then referred to as reactions.
In a same way, all solicited systemic events pre-listed in the CRF are also considered as related to study vaccination and will be considered as reactions.

For unsolicited systemic AE, missing relationship to the study vaccine (investigation product, including VRVg-2, Verorab and Imovax Rabies) in eCRF will be considered as related to study vaccine at the time of analysis.

Note: Due to the relationship to HRIG (non-investigation product) is not collected in eCRF, any AEs related to HRIG (including those occurring at HRIG injection location) will be reported as unsolicited systemic events and the relationship to HRIG will not be analyzed. In consequence, any AE considered as causally related to HRIG but not related to study vaccine will be analyzed as unsolicited systemic AE unrelated to study vaccine.

The missing relationship to study procedures for SAEs will not be imputed.

4.3.2.3 Measurements

Partially missing temperatures will be handled as described in Section 3.3.1.1.1.

4.3.2.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 3.3.1.1.1. For unsolicited AEs, missing intensities will remain missing and will not be imputed.

4.3.2.5 Start Date and Stop Date

Missing or partially missing start dates for unsolicited AEs will remain missing and not be imputed.

If either the start is missing or partially missing, the time of onset will be considered as missing.

Nevertheless, unsolicited AEs with missing time of onset will be included in analyses according to the visit collected.

Missing or partially missing stop dates for AEs (solicited reactions and unsolicited AEs) will remain missing and not be imputed.

4.3.2.6 Action Taken

Missing actions taken will remain missing and not be imputed.

4.3.3 Efficacy

Not applicable.

4.4 Interim / Preliminary Analysis

No formal interim analyses are planned; the statistical analysis will be performed in 2 steps as follows: a first statistical analysis of the safety and immunogenicity data obtained up to D56 will be conducted once data are available and an interim database lock will be conducted. A final analysis will be conducted once the 6-month safety data have been collected and the final database lock has occurred.

No statistical adjustment is necessary because there are no repeated analyses of the same parameter.

4.5 Determination of Sample Size and Power Calculation

The sample size calculation is driven by the non-inferiority testing.

An alpha level of 2.5% (one-sided hypothesis), a maximum acceptable difference of 5% for the proportion of subjects with an RVNA titer ≥ 0.5 IU/mL at D28, and a power of at least 90% for each of the non-inferiority testing have been chosen to calculate the sample size, in order to ensure an overall power of at least 80% to reach the primary objective. Assuming a proportion of subjects with an RVNA titer ≥ 0.5 IU/mL of 99% for the 3 vaccine groups, and an unbalanced randomization 3:1:1 (VRVg-2 + HRIG: Verorab vaccine + HRIG: Imovax Rabies vaccine + HRIG), 213 subjects in the VRVg-2 + HRIG group and 71 subjects in each of the Verorab vaccine + HRIG and Imovax Rabies vaccine + HRIG groups will be necessary, to provide a power of 80% to test the global null hypothesis, using the Farrington and Manning method (4).

Originally, it was planned to enroll 504 subjects in this study. Following a clinical hold between Friday, 06 September 2019 and Tuesday, 10 September 2019, for the evaluation of an SAE as required by the ANSM, the sample size of the study had been modified to replace subjects who were to be excluded from the PPAS due to vaccination outside of the recommended time window and to increase the attrition rate of the study.

Under the assumption that 20% of subjects will not be evaluable, 267 subjects in the VRVg-2 + HRIG group and 89 subjects in each, the Verorab vaccine + HRIG and the Imovax Rabies vaccine + HRIG groups should be enrolled.

As a consequence of the first clinical hold of the study, 71 subjects from the 4 vaccine groups (assumed to be distributed with a distribution close to 36:12:12:12) were identified as to be excluded from the PPAS. With the aim to secure the study power, 60 additional subjects were to be enrolled among the 3 vaccine groups involved in the primary objective assessment, ie, a total of 606 subjects were to be enrolled as follows: 303 in the VRVg-2 + HRIG group and 101 in each of the Verorab vaccine + HRIG and Imovax Rabies vaccine + HRIG groups.

Then, in the context of the COVID-19 crisis, enrollment was paused from the 12th of March 2020 and ongoing vaccination were stopped on the 16th of March 2020. Twenty-nine ongoing subjects (from all the 4 groups) are expected to be withdrawn and excluded from the PPAS as they are unable to complete their vaccination schedule as planned in the protocol due to national confinement measures. Still with the aim to secure the study power and maintain the randomization ratio, 30 additional subjects will be enrolled in the study. Finally, 636 subjects are

planned to be enrolled, with a planned overall distribution of 318:106:106:106 among the 4 vaccine groups.

Assuming a proportion of subjects with an RVNA titer ≥ 0.5 IU/mL of 99% in the VRVg-2 + HRIG group, this sample size will give more than 93% of power to demonstrate the superiority of this rate to 95% on the PPAS.

In order to maintain the 3:1:1:1 ratio, the additional arm of subjects receiving VRVg-2 as standalone will include 106 subjects; the results will be descriptive, mainly versus the VRVg-2 + HRIG group. The number of subjects is the same as the 2 controls to facilitate group allocation, and will allow good precision of the results.

The 3:1:1 design is chosen to optimize the non-inferiority testing for immunogenicity with 2 comparators and to increase the size of the safety database of VRVg-2 vaccine.

In case of any new study hold, withdrawn or out of vaccination time windows subjects might be replaced and sample size might be increased accordingly.

4.6 Data Review for Statistical Purposes

A treatment blind review of the data has been anticipated through the data review process led by Data Management before database lock. This review of the data included a statistical review.

4.7 Changes in the Conduct of the Trial or Planned Analyses

No significant change occurred during the conduct of the trial not documented in a protocol amendment.

5 References List

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4 Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Stat Med. 1990;9(12):1447-54.