NCT03145766

Immunogenicity and Safety of a Purified Vero Rabies Vaccine - Serum Free when administered according to a Simulated Rabies Post-exposure Regime in Healthy Adults

Multicenter, observer-blind, controlled, randomized, Phase II study in 320 subjects aged 18 to <65 years. Each subject will receive one of the 2 formulations of the purified Vero Rabies - Serum Free vaccine (VRVg) or the Human Dipoid Cell Vaccine Imovax® Rabies. Vaccination will be according to the ESSEN post-exposure regimen and human rabies immunoglobulins (HRIG) will be administered at D0.

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	VRV11
Development Phase:	Phase II
Sponsor:	Sanofi Pasteur SA, 2, avenue Pont Pasteur, F-69367 Lyon cedex 07, France
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 1.0 17 JUL 2017

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List of Abbreviations

AE	adverse event
AESI	adverse event of special interest
AR	adverse reaction
BL	blood sample
CI	confidence interval
CSR	clinical study report
D	Day
DC	diary card
eCRF	electronic Case Report Form
FAS	full analysis set
GM	geometric mean
GMT	geometric mean titer
GMTR	geometric mean titer ratio
HDCV	human diploid cell vaccine
HRIG	Human rabies immunoglobulins
IU	international unit
LLOQ	lower limit of quantitation
M	month
MedDRA	Medical Dictionary for Regulatory Activities
MD	missing data
mL	milliliter
MA	memory aid
NM	not measurable
PPAS	Per-Protocol analysis set
PT	preferred term
PVRV	purified Vero rabies vaccine
Q1	the first quartile
Q3	the 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
TLF	table listing figure
ULOQ	Upper limit of quantitation
V	Visit
Vac	vaccination
WHO	World Health Organization

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1 Introduction

Currently, Sanofi Pasteur is the license holder of two rabies vaccines on the market worldwide: Imovax® Rabies and Verorab®. Imovax® Rabies, a human diploid cell vaccine (HDCV), was licensed in 1975 in France and is registered in the USA since 1980. Verorab®, a Purified Vero Rabies vaccine (PVRV), was first licensed in 1985 in France. Both vaccines have well-defined safety and immunogenicity profiles.

The Sponsor has improved the current Verorab® in order to develop a new generation of rabies vaccine, highly purified and produced without Human or Animal origin raw material: the Purified Vero Rabies Vaccine - Serum Free, henceforth referred to as VRVg. VRVg is compliant with standards set by the European Pharmacopoeia, World Health Organization (WHO) and US Pharmacopeia.
VRVg has been evaluated in 5 clinical trials to date: VRV01, VRV08, VRV04, VRV02 and VRV06. [redacted] Sanofi Pasteur made the decision to modify the VRVg initial formulation (referred to as VRVg-1) [redacted]. The modified formulation, referred to as VRVg-2, [redacted]
A phase II study (VRV11) using the most stringent settings is proposed to secure the selection of the final dosage before embarking in larger phase III non inferiority studies.

2 Trial Objectives

Objectives

Immunogenicity

- To describe the immune response induced by VRVg-2 (low, medium and high dosage) versus VRVg-1 and Imovax Rabies, at D14 (7 days after the third injection), at D28 (14 days after the fourth injection), at D42 (14 days after the last injection), and at M7 (6 months after the last injection).

Safety

- To describe the clinical safety profile of VRVg-2 (low, medium and high dosage) versus VRVg-1 and Imovax Rabies after each vaccine injection.

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

This will be a multicenter, observer-blind, controlled, randomized, Phase II study.

A total of 320 subjects aged 18 to <65 years will be included in the study.

Each subject will receive one of the 2 formulations of the Purified Vero Rabies Vaccine - Serum Free (VRVg) or the Rabies Humain Diploid Cell Vaccine Imovax Rabies. Vaccination will be according to the ESSEN post-exposure schedule (i.e., a total of 5 injections [1 injection per day] administered at D0, D3, D7, D14 and D28) and human rabies immunoglobulins (HRIG) will be administered at D0. Subjects will be assigned to the VRVg-2 groups (low, medium and high dosage), VRVg-1 group, or Imovax Rabies group with a 2:2:2:1:1 allocation ratio..

3.2 Trial Plan

The trial plan is summarized in Table 3.2.

Blood sampling:

Immunogenicity will be assessed in all subjects included in the trial, before the first vaccine injection (VAC1) (D0), before VAC4 (D14), before VAC5 (D28), 14 days after VAC5 (D42) and 6 month after VAC5 (M7). The rapid fluorescent focus inhibition test (RFFIT) assay will be used for immunogenicity assessment.

All subjects will provide a total of five 5-mL blood samples to assess the immune response induced by the rabies vaccines.

Table 3.1 below outlines the schedule of blood sampling and vaccine injection.

Table 3.1: Blood Sampling and Vaccination Schedule

	V01	V02	V03	V04	V05	V06	V07	V08
Visit interval	VAC1	VAC2= VAC1+3D	VAC3 = VAC1+7D	VAC4 = VAC1+14D	VAC5= VAC1+28D	VAC5+14D	VAC5+28D	VAC5+6M
Vaccine injection	x	x	x	x	x			
HRIG (Imogam® Rabies)	x							
Blood sampling (5 mL)	x			x	x	x		x

Collection of safety data:

Subjects will be observed for safety for 30 minutes after each vaccination, and any adverse events (AE)/reaction occurring between each vaccination, and up to 28 days after the fifth vaccination will be recorded by the subjects in a diary card (DC).

At each visit, study staff will either check and/or collect the safety DCs provided to subjects to report safety data. A memory aid (MA) will be provided to the subjects at V07 to collect serious adverse events (SAEs) and adverse events of special interest (AESIs) up to the end of the study. The MA will be checked and collected at V08.

Table 3.2: Trial with 8 Visits, 5 Vaccinations, 5 Blood Samples, 7 Months Period/Subject

Visit	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**
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	√							
Demography	√							
Urine pregnancy test *	√	√	√	√	√			
Physical examination	√	√	√	√	√	√	√	
Past and current significant medical history	√							
Inclusion & exclusion criteria	√							
Randomization	√							
Blood sampling for serology (5 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 MA
Injection site reactions and Systemic Event Assessment	√	√	√	√	√	√	√	
Temporary contraindications		√	√	√	√			
Reportable concomitant medication	√	√	√	√	√	√	√	
Termination Record								√
Definitive contraindications	Collected throughout the trial or up to V05							
Pregnancy cases	Collected throughout the trial							
SAEs and AESIs	Collected throughout the trial							

* For subjects of childbearing potential

4 Endpoints and Assessment Methods

4.1 Immunogenicity Endpoints and Assessment Methods

See Section 9.1 of the protocol.

4.2 Safety Endpoints and Assessment Methods

See Section 9.2 of the protocol.

4.3 Derived Endpoints: Calculation Methods

4.3.1 Safety

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

- AE: Adverse event includes immediate, solicited, and unsolicited non-serious, or serious event
- AR: Adverse reaction corresponds to related AE
- Immediate: Unsolicited systemic non-serious AE ticked "immediate (within 30 minutes from the vaccination)" by the investigator in the electronic case report form (eCRF) or SAE with time of onset within 30 minutes
- Solicited reaction: Event pre-listed in the eCRF, and which occurred during the solicited period
- Unsolicited AE: AE recorded in the eCRF as unsolicited or SAE forms, excluding solicited reaction. Therefore, include immediate AE.

  Unsolicited non-serious injection site reactions are always recorded without relationship and analyzed as ARs.

  Unsolicited AEs analyzed are AEs up to 28 days +3 days after vaccination. Unsolicited AEs occurring after the defined period - and which are not SAEs - will be presented in a separate listing.
- SAE: Unsolicited AE considered serious by the investigator (reconciled with Global Pharmacovigilance database)
- Adverse event of specific interest (AESI): the list of Medical Dictionary for Regulatory Activities (MedDRA) preferred terms (PT) defined in the protocol to select these AEs in the database:
  - Anaphylactic reaction (PT)
  - Encephalitis (PT)

- Convulsion (PT)

4.3.1.1 Solicited Reactions

4.3.1.1.1 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.

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

1) For solicited reactions (except for fever) with an Investigator presence recorded as “No” and with all daily records missing, all daily intensities will be derived as "None."

2) 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 (e.g., swelling measurement > 0 mm but < 25 mm in adults).

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.

4.3.1.1.2 Maximum Overall Intensity

Maximum overall intensity is derived from the daily intensities computed as described in Section 4.3.1.1.1 and is calculated as the maximum of the daily intensities over the period considered.

4.3.1.1.3 Presence

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

- None: no presence
- Grade 1, Grade 2, or Grade 3: presence
- Missing: missing presence

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

4.3.1.1.4 Time of onset

Time of onset is derived from the daily intensities computed as described in Section 4.3.1.1.1. It corresponds to the first day with intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (i.e., 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.

4.3.1.1.5 Number of Days of Occurrence

Number of days of occurrence over the period considered is derived from the daily intensities computed as described in Section 4.3.1.1.1. 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.

4.3.1.1.6 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 occurrence 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 occurrence is:

- (stop date – last vaccination date) + (number of days of occurrence 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".

4.3.1.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed 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. Note the intensity could be considered None (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement > 0 mm but < 25 mm in adults).If the last daily intensity of the solicited period is at least Grade 1 and maximum intensity on the ongoing period is also at least Grade 1, then the reaction is considered "ongoing". In any other cases the reaction will not be considered as "ongoing".

4.3.1.2 Unsolicited Non-serious Adverse Events (AEs)

4.3.1.2.1 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 be included in the listing "Unsolicited non-serious adverse events not included in the safety analysis."

4.3.1.2.2 Intensity

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

If the unsolicited non-serious AE is measurable and its preferred term (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 that the intensity could be considered "None" (not a reaction) in the analysis despite being

considered a reaction by the investigator (e.g., swelling measurement > 0 mm but < 25 mm in adults).

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

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

4.3.1.2.3 Last Vaccination

Last vaccination before an unsolicited non-serious AE is derived from the visit numbers provided in the clinical data base and is calculated as follows:

- If an unsolicited non-serious AE has a non-missing visit number, the visit number should be used to determine the last vaccination before the unsolicited non-serious AE
- If the visit number is missing, then the start date should be used to determine the last vaccination before the unsolicited non-serious AE.

4.3.1.2.4 Time of onset

Time of onset is derived from the start date of the unsolicited non-serious AE provided in the clinical database and the date of last vaccination:

- start date of the unsolicited non-serious AE – date of previous vaccination

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

An AE with missing time of onset will be considered to have occurred just after the vaccination indicated by the visit number, so will be included in these tables. Note: Unsolicited non-serious AE that occurred before vaccination (negative time of onset) will not be included in analysis, but will be listed separately.

4.3.1.2.5 Duration

Duration is derived from the start and stop dates of the unsolicited non-serious AE provided in the clinical database:

- stop date of unsolicited non-serious AE - start date of unsolicited non-serious AE + 1.

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

4.3.1.3 SAEs

4.3.1.3.1 Last Vaccination

Last vaccination before an SAE will use the same methodology as the last vaccination before an unsolicited non-serious AE as described in Section 4.3.1.2.4.

4.3.1.3.2 Time of onset

Time of onset will be computed using the same methodology than for unsolicited non-serious AEs as described in Section 4.3.1.2.5.

4.3.1.3.3 Duration

Duration will be computed using the same methodology than for unsolicited non-serious AEs as described in Section 4.3.1.2.5.

4.3.2 Immunogenicity

4.3.2.1 Computed Values for Analysis

[redacted]

4.3.3 Efficacy

Not applicable.

4.3.4 Derived Other Variables

4.3.4.1 Duration of a Subject in the Trial

The duration of a subject in the study is computed as follows: maximum (date of visit, date of termination form) − V01 date +1.

4.3.4.2 Duration of the Trial

The duration of the study is computed as follows: maximum of all subjects (date of visit, date of termination form) – minimum for all subjects (date of V01) +1.

4.3.4.3 Age calculation

The age of a subject in the study is computed as follows:

Age in years: (Date of 1st injection> - Date of birth +1) / 365.25

Following age groups will be derived:

18 to 40 years: from 18th birthday date to the last day before the 41st birthday date

41 to <65 years: from 41st birthday date to the last day before the 65th birthday date

5 Statistical Methods and Determination of Sample Size

The statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics platform using SAS® Version 9.4 software or later.

The statistical analysis will be performed in 2 steps: first, all the data collected on the period D0-D56 will be analyzed, and then the data up to M7 will be added to the first statistical report.

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 5.1: Descriptive Statistics Produced

Baseline characteristics and follow-up description	**Categorical data**	Number of subjects. Percentage of subjects.
	Continuous data	Mean, standard deviation, quartiles, minimum, and maximum.
Clinical safety results	**Categorical data**	Solicited: Number and percentage (95% CIs) of subjects. Unsolicited: Number and percentage (95% CIs) of subjects, and number of events.
Immunogenicity results	**Categorical data (cutoff)**	Number and percentage (95% CIs) 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, quartiles, minimum, and maximum. Graphical representation by Reverse Cumulative Distribution Curve (RCDC).

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

For immunogenicity results, assuming that Log_{10} 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 geometric means (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.

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Immunogenicity Objective

5.1.1.1 Hypotheses

No hypothesis will be tested for the immunogenicity objective.

5.1.1.2 Statistical Methods

5.1.1.2.1 Immunogenicity

The descriptive immunogenicity analysis at D0, D14, D28, D42 and M7 will be performed to document immunogenicity of the 5 groups.

5.1.1.2.1.1 Descriptive Immunogenicity

For all the subjects, a descriptive analysis for pre dose 1 (D0), pre dose 4 (D14), pre dose 5 (D28), 14 days post dose 5 (D42) and 6 months after the last injection (M7) will present the following statistics for the rabies virus neutralizing antibody (RVNA) titers in each randomized vaccine group:

- Number of subjects with RVNA titer available
- Number and percentage of subjects with RVNA titer ≥ 0.2 (LLOQ) IU/mL 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 neutralization at the starting dilution (1/5) of the RFFIT assay
- GMTs and 95% CI of RVNA titers
- GM of individual titer ratio (GMTR):
  - D14/D0
  - D28/D0
  - D42/D0

- M7/D0

- Distribution of titers (minimum, Q1, median, Q3, maximum)
- Log_{10}: mean and its standard deviation (SD)
- Ratio of GMT at D14, D28 and D42 between:
  - Each VRVg group (VRVg-2 low, medium and high dosages and VRVg- 1 group) versus Imovax® Rabies
  - Each VRVg-2 group (VRVg-2 low, medium and high dosages) versus VRVg-1
  - VRVg-2 medium and high dosages versus low dosage
  - VRVg-2 high dosage versus medium dosage

The lower and upper limits of the two-sided 95% CIs of the proportions will be computed using the exact binomial method (Clopper-Pearson method) (1). For the ratio of GMTs between VRVg and Imovax® Rabies, the 95% CI will be obtained by antilog back-transformations of the CI of the difference [$log_{10}(GMT_{VRVg})$-$log_{10}(GMT_{Imovax\ Rabies})$] obtained using the usual calculation for normal distribution.

The complete descriptive immunogenicity data will be analyzed according to randomization group in FAS population. The main parameters, defined as GMTs and percentages of subjects with titers ≥ 0.5 IU/mL will also be described on the PP population.

Additionally, RCDC will be plotted in all vaccine groups, at D0, D14, D28, D42 and M7. Boxplots and density distribution of RVNA titers at D14, D28 and D42 will also be produced. All these figures will be plotted on the FAS population.

5.1.2 Hypotheses and Statistical Methods for Safety Objective

5.1.2.1 Hypotheses

No hypothesis will be tested for the safety objective.

5.1.2.2 Statistical Methods

5.1.2.2.1 Safety

The analysis will be descriptive. The statistics presented on Table 5.1 will be produced.

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.

- Solicited and unsolicited injection site reactions will be presented up to 7 days after each injection.

- Solicited systemic reactions will be collected between the first and the second injection, between the second and the third injection, and up to 7 days after the remaining injections.
- Unsolicited events will be presented between each injection and up to 28 days after the last injection.
- SAEs throughout the trial.
- AESIs throughout the trial.

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.
- 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).

As exploratory analyses, the possible influence on the safety results of covariates such as age group (18 to 40 years and 41 to <65 years), gender, race, and center will be evaluated.

Solicited Reactions

The solicited injection site reactions and the solicited systemic reactions will be presented according to the term listed in the CRF, separately (except in safety overview table).

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 5.2

Table 5.2: 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 5.3

Table 5.3: 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

Solicited reaction after any injection

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

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

- *Overall occurrence over the D0-D7 period for injection site reactions or between vaccinations for systemic reactions (up to D7 for the last 2 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 on-going solicited reactions.*

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 nature (SOC and PT of the MedDRA classification) and will be listed.

Unsolicited Adverse Events

The unsolicited non-serious 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 †

† For subjects interrupting their vaccination schedule after the first injection, unsolicited AEs until 28 days after the first injection are collected at visit V02

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

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

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

- "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

An AE reported with an onset > 28+3 days after an injection will not be included in the analysis, but will be listed separately. 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 vaccine injection as assessed by the investigator), time of onset, duration and maximum intensity.

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

- *Maximum intensity:*

  - Missing
  - Grade 1
  - Grade 2
  - Grade 3

- *Time of onset categories*

  - Missing
  - D0-D3
  - D4-D7
  - D8-D14
  - >=D15

‡ For subjects interrupting their vaccination schedule after the second injection, unsolicited AEs until 28 days after the second injection are collected at visit V03

§ For subjects interrupting their vaccination schedule after the third injection, unsolicited AEs until 28 days after the first injection are collected at visit V04

** For subjects interrupting their vaccination schedule after the fourth injection, unsolicited AEs until 28 days after the second injection are collected at visit V05

- *Maximum duration:*

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

SAEs and AESIs

The SAEs and AESIs occurred during the study will be analyzed on the following periods:

1. vaccination period: after any vaccination period (D00-D56[+3D])
2. during the six-month safety follow-up period (all SAEs and AESIs from D56[+3D])

SAEs will be analyzed according to their causality (relationship to vaccine injection as assessed by the investigator), seriousness, 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.

5.1.3 Exploratory analyses

5.1.3.1 5.1.3.1 Immunogenicity

5.1.3.1.1 Descriptive immunogenicity in the subset of subjects with a baseline titer < 0.5 IU/mL

In case more than 5% of subjects in the FAS (all vaccine groups) have a baseline titer ≥ 0.5 IU/mL, a descriptive analysis of immunogenicity parameters (GMT and percentage of subjects with a titer >0.2 IU/mL at D0, D14, and D42) will be provided on the subset of subjects with a baseline titer <0.5 IU/mL. This descriptive immunogenicity analysis will be performed according to the randomized vaccine on the FAS population.

5.1.3.1.2 Covariate Exploration of the Immunogenicity Results

The possible influence on the immune response at D14, D28 and D42 of the following characteristics will be studied:

1) Gender
2) Age "18 to 40 years" and "41 to <65 years"
3) Race (only if more than 5% of subjects in several race categories)

4) Center

A descriptive table simplified to the most relevant immunogenicity parameters will be provided for each sub-population, e.g. males and females. A homogeneity Fisher test will be performed to compare the proportion of subject with an RVNA titer ≥ 0.5 IU/mL of each subpopulation inside each vaccine group. The rationale for the proposed test is to provide evidence of whether or not, some differences can be observed between subpopulations on GMTs and on the proportion of subjects with an RVNA titer ≥ 0.5 IU/mL.

These covariate analyses will be performed on the FAS population.

5.2 Analysis Sets

Three main analysis sets will be used: the Full Analysis Set, the Per-Protocol Analysis Set, and the Safety Analysis Set.

5.2.1 Full Analysis Set

The FAS is defined as the subset of randomized subjects who received at least one dose of the study vaccine.

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

5.2.2 Per-Protocol Analysis Set

The per-protocol (PP) analysis set is a subset of the full analysis set (FAS).

The PPAS will be defined based on the D14 timepoint (in healthy individuals who are vaccinated, the 0.5 IU/mL threshold considered to be protective should be achieved in most individuals by day 14 after the first injection of a post-exposure regimen, with or without simultaneous administration of rabies) (2).

The subjects presenting with at least one of the following relevant protocol deviations before D14 (i.e. 7 days after the third vaccine injection) will be excluded from the PPAS:

- Subject did not meet all inclusion protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not receive the correct number of the first three doses of vaccine
- Subject received a vaccine other than the one that he/she was randomized to receive for the first three doses of vaccine
- Preparation and/or administration of vaccine was not done as per protocol for the first three vaccinations
- Subject did not receive 2nd and 3rd vaccines in the proper time window from D0, as follows:
  - Vac2 in [D3-D5]

  - Vac3 in [D7-D9]
- Subject did not provide a post-dose 3 serology sample in the proper time window [D13-D15] from D0
- Subject received a protocol-restricted therapy/medication/vaccine (belonging to category 2) from D0 to D14
- Subject's serology sample is missing or did not produce valid test results at both D0 and D14
- Seropositive subject at D0, i.e. RVNA titer ≥ LLOQ
- Subject developed a protocol-specified withdrawal criterion from D0 to D14 but was not withdrawn
- Subject did not receive the correct amount of Imogam at D0
  - Outside the +/-10% the theoretical calculated volume

The PP analysis set will be defined at D14. Adherence to the definition of the PP analysis set may be decided during a blind-review of data, i.e. before breaking the code. Subjects in the PP analysis set will be analyzed according to the vaccine received.

5.2.3 Safety Analysis Set

The safety analysis set (SafAS) is defined for each dose as the subset of subjects having received this dose. Any subject who receives a dose will be analyzed according to the treatment received at this dose and after any dose according to the vaccine 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 vaccine at a given visit or if the vaccine received does not correspond to any protocol group at a specific dose, the subject is excluded from the SafAS at this dose, however, this 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.

5.2.4 Populations Used in Analyses

Immunogenicity analyses will be performed on the FAS, according to the randomized vaccine group. The main immunogenicity analyses will also be performed on the PPAS, according to the injected group.

Safety analyses will be performed on the SafAS, according to the injected vaccine group.

5.3 Handling of Missing Data and Outliers

5.3.1 Immunogenicity

No replacement will be done as the degree of missing data is expected to be very low.

5.3.2 Safety

No replacement will be done as the degree of missing safety data is expected to be very low.

Missing data in vaccine studies are mostly due to dropouts. The dropouts due to AEs or lost to follow-up subjects will be identified and discussed in the study report.

5.3.2.1 Immediate

For unsolicited non-serious 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.

For SAEs, missing or partially missing elapsed time from last injection (recorded if within 24 hours) will remain missing and will not be imputed.

5.3.2.2 Causality

Missing causality (relationship) for unsolicited non-serious AEs and SAEs will be considered at the time of analysis as related to vaccination.

5.3.2.3 Measurements

Partially missing temperatures will be handled as described in Section 4.3.1.1.1.

5.3.2.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.3.1.1.1.

5.3.2.5 Start Date and Stop Date

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

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

If either the start or stop date is missing or partially missing, the time of onset will be considered to be missing. Nevertheless unsolicited AEs with missing time of onset will be included in analyses according to the visit collected.

5.3.3 Efficacy

Not applicable.

5.4 Interim/Preliminary Analysis

No formal interim analyses are planned.

The statistical analysis will be performed in two steps. A first statistical analysis will be performed when results on all immunogenicity and safety data collected up to D56 (i.e., up to 28

days after the primary vaccination series) will be obtained and locked. A final statistical analysis will be performed when immunogenicity and safety results from D56 to M7 will be obtained and locked.

5.5 Determination of Sample Size and Power Calculation

An arbitrary number of 80 subjects per VRVg-2 group and 40 subjects in both VRVg-1 and Imovax Rabies has been proposed.

[redacted]

Table 5.4: [redacted]

[redacted]

In terms of safety, according to the occurrence rate of the observed AEs, 80 subjects will allow for the following 95% CI (Table 5.5).

95% CI calculations corresponding to anticipated AEs rates in VRVg-2 groups of 80 subjects.

Table 5.5: 95% CI calculations corresponding to anticipated AEs rates in VRVg-2 groups of 80 subjects

Observed numbers	Observed %	95% CI
8/80	10.0	(4.4; 18.8)
12/80	15.0	(8.0; 24.7)
16/80	20.0	(11.9; 30.4)
20/80	25.0	(16.0; 35.9)
24/80	30.0	(20.3; 41.3)

5.6 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.

5.7 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.

6 References List

1 Newcombe R.G., Two-sided confidence intervals for the single proportion: comparison of seven methods, Statistics in Medicine, (1998) 17, 857-872

2 Rabies Vaccines: WHO Position Paper; Weekly Epidemiological Record, No 32, 2010, 85, 309-320

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