SAFETY AND IMMUNOGENICITY OF A NEXT GENERATION PURIFIED VERO RABIES VACCINE AS A SIMULATED INTRADERMAL POST-EXPOSURE PROPHYLAXIS IN ADULTS AND CHILDREN IN THAILAND: A PHASE 3, RANDOMIZED STUDY

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Abstract. The aim of this Phase 3, randomized study was to assess the immunogenicity and safety of a purified Vero cell rabies vaccine, PVRV-NG2, compared with the current rabies standard of care vaccine (PVRV), using a simulated post-exposure prophylaxis regimen with intradermal (ID) vaccination on day (D) 0, D3, D7, and D28 in healthy pediatric (≥1 to <18 years of age) and adult (≥18 years of age) participants, with concomitant administration of rabies immunoglobulin [RIG]). Rabies virus neutralizing antibody (RVNA) titers were determined on D0, D14, D42, and D90. Enrolled participants (n = 402) consisted of pediatrics (n = 168) divided into two groups, Group 1 receiving PVRV-NG2 (n = 112) and Group 2 receiving PVRV (n = 56), and of adults (n = 234) divided into 4 groups, Group 3 receiving PVRV-NG2+equine RIG (ERIG, n = 26), Group 4 receiving PVRV+ERIG (n = 14), Group 5 receiving PVRV-NG2+human RIG (HRIG, n = 129), and Group 6 receiving PVRV+HRIG (n = 65). By D14, nearly all pediatric participants achieved RVNA titer ≥0.5 IU/ml, while only 52-75% of adults achieved this titer when both vaccines were co-administered with RIGs. By D42, 96 and 100% of adults who received PVRV-NG2 and PVRV respectively, had RVNA titers ≥0.5 IU/ml. By D90, all, except two, pediatric participants had RVNA titers that persisted at ≥0.5 IU/ml, while 75 and 78%

of adults who received PVRV-NG2 and PVRV respectively, had maintained this titer. No safety concerns were identified, and safety profiles were similar across groups. Overall, the immunogenicity and safety profiles of PVRV-NG2 when administered alone or co-administered with HRIG were comparable with those of PVRV, supporting the application of intradermal administration for post-exposure vaccination using the updated Thai Red Cross vaccination schedule (Clinicaltrials.gov no: NCT04478084).

Keywords: adult, intradermal vaccination, pediatric, PEP, PVRV-NG2, rabies

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INTRODUCTION

Rabies causes about 59,000 human deaths annually worldwide, the majority of which occur in children under 15 years of age in poor and rural areas of Africa and Asia (Knobel *et al*, 2005; Hampson *et al*, 2015; Durrheim, 2017), although, owing to the widespread under-reporting of rabies cases, the true death toll is probably much higher. Rabies is endemic in Thailand, with 40% of those bitten by suspected rabid animals being children (WHO, 2024a). Surveillance data showed fatalities across Thailand peaking at 18 fatal cases in 2018 (WHO, 2024b). In Thailand, children have the highest exposure rate, with 189.7 exposures/100,000 population, compared with other age groups (Jane Ling *et al*, 2023).

Effective rabies prevention relies on increasing awareness of the disease, enhancing access to vaccines for high-risk populations, ensuring timely wound care management in the event of exposure, and limiting rabies in domestic and wild animals through control efforts including animal vaccination programs. Therefore, access to effective rabies vaccines and immunoglobulins is central to rabies management.

Multiple rabies vaccines are available globally; these are prepared using different substrates, such as human tissue culture (human diploid cell vaccine [HDCV]), primate tissue

cultures (purified Vero cell vaccine [PVRV]) or avian tissue (purified chick embryo cell culture vaccine [PCECV]). Currently, Sanofi has two standard-of-care vaccines available, HDCV (Imovax rabies®; Sanofi, Lyon, France) or PVRV (Verorab®; Sanofi, Lyon, France), both of which have well-defined safety and immunogenicity profiles (Plotkin, 2000; Toovey, 2007). However, despite the availability of multiple rabies vaccines, access is not always secured owing to supply issues (Abela-Ridder *et al*, 2016).

A new generation PVRV vaccine (PVRV-NG) was developed, prepared from Wistar Rabies Pitman Moore/WI 38 1503-3M strain. The PVRV-NG vaccine has an extremely low residual DNA content and is manufactured without raw materials of human or animal origin or antibiotics, which is consistent with the World Health Organization (WHO) and European Medicines Agency recommendations (WHO, 2003; EMA, 2011). Two candidate PVRV-NG formulations were developed: PVRV-NG2 contains increased antigen content compared to PVRV-NG and displayed similar immunogenicity and safety to HDCV, and was therefore selected for evaluation in Phase 3 trials (Pichon *et al*, 2023; Pineda-Peña *et al*, 2024).

Intradermal (ID) administration of rabies vaccine is supported by the WHO and has been implemented in multiple countries (WHO, 2018b). When administered via the ID route, current vaccines have shown comparable immunogenicity with the same vaccine administered via the intramuscular (IM) route (WHO, 2018b; Denis *et al*, 2019; Moulenat *et al*, 2020). ID administration has several advantages over IM administration. For example, IM administration requires an entire vial of vaccine, whereas ID administration requires only a fraction of the vial's content, allowing a vial to be used among several vaccine recipients, thereby increasing vaccine availability (Denis *et al*, 2019). In addition, despite a lower amount of rabies antigen being injected via the ID route, the high concentration of antigen-presenting cells in the dermis is expected to provide a strong immunologic response (WHO, 2017; WHO, 2018b). Furthermore, ID administration reduces the cost of vaccination by 60-80% compared to an IM schedule for the same vaccine (Tarantola *et al*, 2015; Salahuddin *et al*, 2016; Denis *et al*, 2019).

This study aimed to describe the immunogenicity and safety profiles of PVRV-NG2 in healthy pediatric (≥1 to <18 years of age) and adult (≥18 years of age) populations in Thailand in comparison with the current rabies standard-of-care vaccine, PVRV, using a simulated ID post-exposure prophylaxis (PEP) regimen. The expected outcome was that PVRV-NG2 would show comparable immunogenicity and safety to PVRV when administered as a simulated ID PEP regimen.

MATERIALS AND METHODS

Study design and participants

A Phase 3, randomized, observer-blinded, controlled, multicenter study was conducted at three study centers in Thailand, namely, the Internal Medicine Department of Srinagarind Hospital in Khon Kaen Province, and the Department of Tropical Pediatrics and the Vaccine Trial Center at the Faculty of Tropical Medicine of Mahidol University in Bangkok, between 5 August 2020 and 21 July 2022. Healthy participants (n = 402) ≥1 year of age were recruited. Exclusion criteria were (i) receipt of any previous pre-exposure prophylaxis (PrEP) or PEP vaccination against rabies; (ii) exposure to a potentially rabid animal within the prior 6 months; (iii) those at high risk for rabies exposure (*eg*, animal handlers, laboratory workers and others in similar risk situations); (iv) receipt of any vaccination in the 4 weeks preceding the first study vaccination or planned day (D) 90; (v) receipt of immunoglobulins, blood or blood-derived products within the previous 3 months; (vi) with known or suspected congenital or acquired immunodeficiency; (vii) receipt of immunosuppressive therapy within the preceding 6 months; (viii) receipt of long-term systemic corticosteroid therapy; (ix) receipt of chloroquine or hydroxychloroquine up to 2 months prior to the study or up to D90 of the study; (x) self-reported thrombocytopenia, bleeding disorders, or receipt of anticoagulants in the 3 weeks prior the study; (xi) known systemic hypersensitivity to any of the vaccine or rabies immunoglobulin (RIG) components, including a positive skin test reaction to equine RIG (ERIG) at visit 1 for the adults enrolled; and (xii) history of a life-threatening reaction to a vaccine containing any of the same substances as the study vaccines.

Vaccination protocol

The test vaccine, PVRV-NG2, and the control vaccine, PVRV, are both purified inactivated rabies vaccines prepared in a Vero cell line in serum-free conditions and provided in freeze-dried form. Each vaccine dose (0.5 ml) contained ≥2.5 IU inactivated rabies virus (Wistar Rabies Pitman Moore/WI 38 1503-3M strain), as per the National Institutes of Health (NIH) potency test (WHO, 2018b). The actual antigen content for all batches was as follows: 8.0 IU/dose for PVRV-NG2 (batch S4497) and 3.25 IU/dose for PVRV (batches R1F31 and T1A79). The antigen content of each vaccine was determined by enzyme-linked immunosorbent assay (ELISA) as previously described (Chabaud-Riou *et al*, 2017; Morgeaux *et al*, 2017).

In addition, the adult study groups received either ERIG (Thai Red Cross Society [TRC], Bangkok, Thailand) provided as a liquid solution in 5-ml vials, with purified equine rabies antiserum at a concentration of 200 IU/ml, or human rabies immunoglobulin (HRIG) (IMOGAM® Rabies-HT; Sanofi, Marcy l'Etoile, France) provided as a liquid solution in 2-ml vials containing 150 IU/ml purified human rabies antiserum. The HRIG potency assessed using a rapid fluorescent focus inhibition test (RFFIT) (Timiryasova *et al*, 2019; Timiryasova *et al*, 2020) was 193 IU/ml.

Eligible participants were randomized in a 2:1 ratio to receive either PVRV-NG2 or PVRV using an Interactive Response Technology, with permuted block randomization stratified by center and age group. Staff, who were not involved in the safety and other study evaluations, prepared and administered the vaccines. Participants, investigators, staff in charge of safety assessments, and sponsor staff were blinded to participant group allocations.

Originally, participants were assigned to the following groups: Group 1, pediatric participants receiving PVRV-NG2; Group 2, pediatric participants receiving PVRV; Group 3, adult participants receiving PVRV-NG2 with concomitant ERIG at D0; and Group 4, adult participants receiving PVRV with concomitant ERIG at D0. However, following reports of serious adverse events (SAEs) of hypersensitivity, the use of ERIG

was discontinued and vaccination of Groups 3 and 4 was halted on 30 September 2020. New participants were recruited and randomized to Group 5 (PVRV-NG2+ HRIG at D0) and Group 6 (PVRV+HRIG at D0).

All participants received a total of four doses (eight injections) of PVRV-NG2 or PVRV, given as two 0.1 ml aliquot ID injections in each arm per vaccination, on D0 (visit 1), D3 (visit 2), D7 (visit 3), and D28 (visit 5) (Fig 1), in line with the vaccination schedule of the Institut Pasteur Cambodge (IPC) updated Thai Red Cross recommendations (WHO, 2018b). ERIG (40 IU/kg) or HRIG (20 IU/kg) was administered IM in the anterolateral thigh at D0 of adult participants only. All participants provided blood samples (3 ml for participants ≥1 to <2 years of age; 5 ml for participants ≥2 to <18 years of age, and 6 ml for adults) prior to the vaccination at D0 (baseline titer), then at D14 (7 days after the third vaccine dose), D42 (14 days after the fourth vaccine dose), and D90 (3 months after the first vaccine dose) (Fig 1). Blood samples were allowed to clot for 1-24 hours (with storage at room temperature for up to 2 hours and refrigeration at 2-8 °C beyond 2 hours) before centrifugation and transfer of the serum into two cryotubes. Serum samples were stored at -20 °C until their shipment on dry ice to the Sanofi Global Clinical Immunology laboratory, Swiftwater, PA, USA, for quantification of rabies virus neutralizing antibodies (RVNA) in serum samples using RFFIT (Smith *et al*, 1973; Timiryasova *et al*, 2019).

Safety was assessed throughout the study. Participants recorded safety information on diary cards throughout the study period (until 6 months post-last vaccination).

Expected outcomes

The primary objective was to describe the immune response induced by PVRV-NG2 and PVRV at D14 (to assess the immune response after three doses) and D42 (to assess the immune response after four doses) when administered as a stand-alone vaccine in a healthy pediatric population (Groups 1 and 2) or when co-administered with HRIG at D0 in healthy adults (Groups 5 and 6). An RVNA titer of 0.5 IU/ml, as recommended by the WHO as a measure of adequate response to vaccination (WHO,

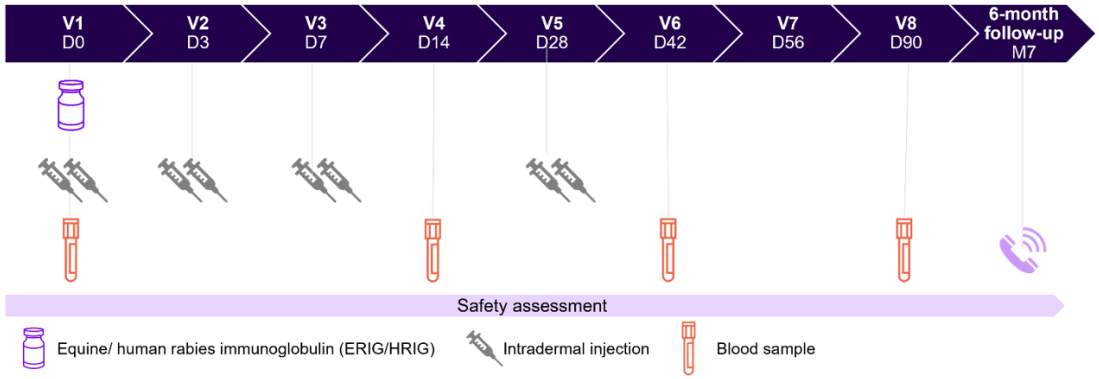

Fig 1 - Study schedule

Study design showing planned visits, injections, blood sample collections, and follow-up. Concomitant administration of equine or human rabies immunoglobulin occurred in adult participants only.

D: day; M: month; V: visit

2018b), and 0.2 IU/ml, the lower level of quantification of the assay, were used in this study as a proxy of complete rabies neutralization (Timiryasova *et al*, 2019). The resulting primary endpoints for the evaluation of immunogenicity were the proportion of participants with RVNA ≥0.5 IU/ml at D0, D14 and D42; the proportion of participants with an RVNA titer ≥0.2 IU/ml (the lower limit of quantification) at D0, D14 and D42; and geometric mean titers (GMTs) at D14 and D42.

The secondary immunogenicity objectives were to describe the immune response induced by PVRV-NG2 and PVRV at D14 after co-administration with ERIG at D0 in healthy adults (Groups 3 and 4) and to describe the immune response induced by PVRV-NG2 and PVRV when administered as a stand-alone vaccine in a pediatric population (Groups 1 and 2) and at D90 after co-administration with HRIG at D0 in healthy adults (Groups 5 and 6). Secondary immunogenicity endpoints were the proportion of participants with an RVNA titer ≥ 0.5 IU/ml at D0 and D14 (Groups 3 and 4) and at D90

(Groups 1, 2, 5, and 6).

The secondary safety objective was to describe the safety profile after each vaccination of PVRV-NG2 and PVRV as stand-alone vaccines in the pediatric population (Groups 1 and 2) and when co-administered with ERIG (Groups 3 and 4) or HRIG (Groups 5 and 6) in adults. The secondary safety endpoints were the occurrences of: (i) unsolicited systemic adverse event (AE) reports in the 30 minutes after each vaccination; (ii) solicited injection-site reactions occurring within 7 days after each vaccination; (iii) solicited systemic reactions occurring between the first and second injections, the second and third injections, and during the 7 days after the remaining injections; (iv) spontaneously reported unsolicited injection-site AEs occurring within 28 days after each vaccination and unsolicited systemic AEs between each vaccination and up to 28 days after the last vaccination; and (v) SAEs and AEs of special interest (AESIs) (anaphylactic reactions, encephalitis and convulsions) throughout the study and up to 6 months after the last vaccination.

Statistical analysis

The sample size was not powered. All immunogenicity and safety analyses were descriptive. Immunogenicity analyses were performed in the per-protocol analysis set (PPAS), the full analysis set for immunogenicity (FASI), and in the full analysis set (FAS), the latter included all randomized participants who received at least one dose of the vaccine. FASI is defined as a subset of the FAS that included all participants from FAS with a baseline RVNA titer <0.5 IU/ml. PPAS for D14 and D42 included all participants in FAS who met all protocol specifications up to D14 and D42 respectively. A safety analysis set (SafAS), which included all participants who received at least one dose of PVRV-NG2 or PVRV, was used for all safety analyses.

For immunogenicity analyses, assuming that the log10 transformation of the titers/data followed a normal distribution, the mean and 95% confidence interval (CI) were calculated as log10 (titers/data), using the Student's t distribution with n-1 degrees of freedom. Antilog transformations

were applied to the results of the calculations to provide geometric means and their 95% CIs. The 95% CIs for the single proportion in immunogenicity and safety results were calculated using the exact binomial method (Clopper-Pearson method) (Newcombe, 1998).

Ethical considerations

The study was conducted in accordance with the protocol and consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and the International Council for Harmonisation for Good Clinical Practice, and all applicable laws, rules and regulations. The study was reviewed and approved by an Independent Ethics Committee or Institutional Review Board of each site. Prior written informed consent was obtained from each participant or parent/legal guardian.

RESULTS

Study design, participants and protocol amendment

Healthy participants (n = 402) were enrolled between 5 August 2020 and 20 December 2021 and randomized among 6 groups as follows: Group 1 [PVRV-NG2, pediatric (n = 112)], Group 2 [PVRV, pediatric (n = 56)], Group 3 [PVRV-NG2+ERIG, adult (n = 26)], Group 4 [PVRV+ERIG, adult (n = 14)], Group 5 [PVRV-NG2+HRIG, adult (n = 129)], and Group 6 [PVRV+HRIG, adult (n = 65)] (Fig 2).

Originally, adult participants (n = 168) were planned to receive either PVRV-NG2+ERIG (Group 3) or PVRV+ERIG (Group 4); however, due to three reports of serious hypersensitivity in adults receiving concomitant ERIG, the sponsor assessed the benefit/risk ratio of the ERIG administration as unfavorable for a simulated PEP regimen in healthy participants. Vaccinations were halted in Groups 3 and 4. Hence, the adult participants randomized to Group 3 (n = 26) and Group 4 (n = 14) were excluded from the PPAS for D42, as they were unable to complete the four-dose vaccination schedule as planned, and 15/40 adult participants were excluded from the PPAS for D14, as they were unable to complete the first three doses of vaccination as planned. Because the protocol allowed replacement of participants who withdrew from the study or

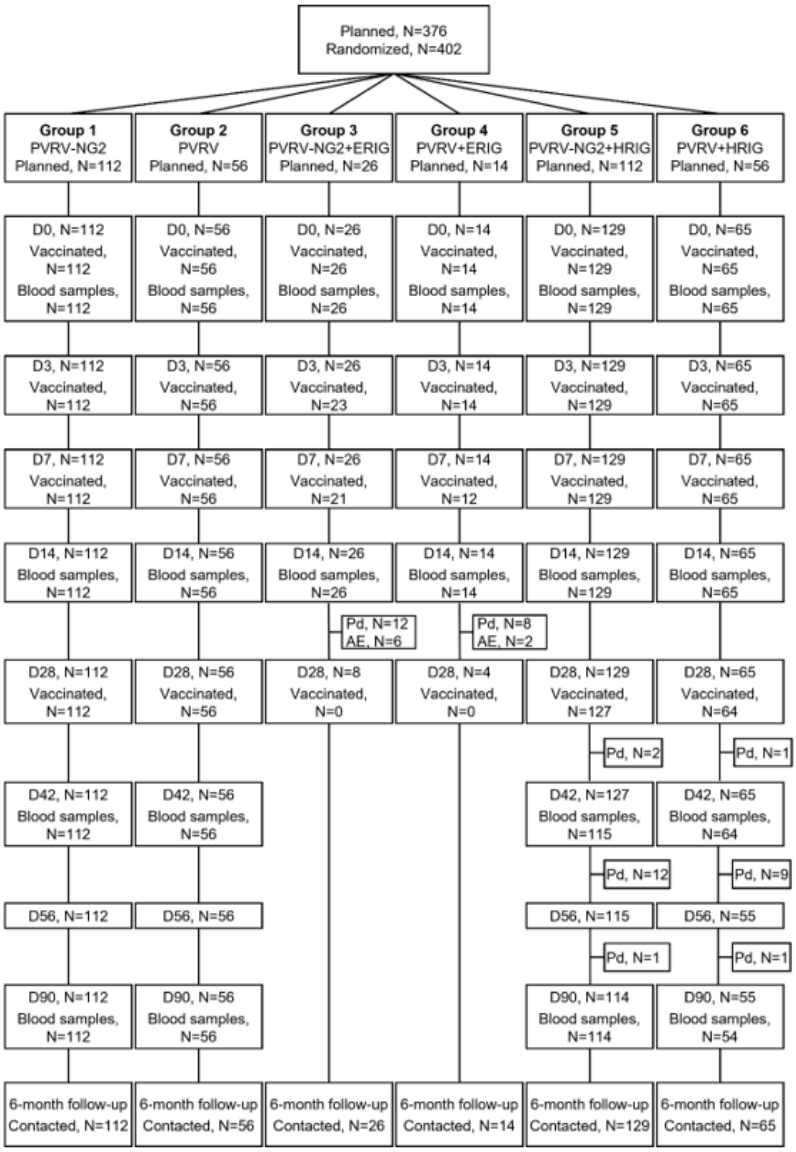

Fig 2 - Participants' disposition flow chart

Groups 1 and 2 included pediatric participants only. Groups 3-6 included adult participants only.

AE: adverse event; D: day; ERIG: equine rabies immunoglobulin; HRIG: human rabies immunoglobulin; Pd: protocol deviation; PVRV: purified Vero rabies vaccine; PVRV-NG2: new generation purified Vero rabies vaccine formulation 2

who were vaccinated outside of the specified time windows, additional participants (n = 26) were allocated to Groups 5 and 6 for the following reasons: received COVID-19 vaccine during the active phase of the study (Group 5, n = 15; Group 6, n = 9), or had already received vaccination against rabies (Group 6, n = 2). As a result, the final adult participants were as follows: Group 3 (n = 40), Group 5 (n = 129) and Group 6 (n = 65) (Fig 2; Table 1).

Among the randomized participants (n = 402), 84% (336/402) participants completed the active phase of the study from D0 to D90 (100% of pediatric participants in Groups 1 and 2, 88% (114/129) of adult participants in Group 5 and 83% (54/65) of adult participants in Group 6) (Fig 2). No adult participants in Groups 3 and 4 completed the active phase due to the termination of the study as described above.

The baseline demographics for the FAS indicated that overall, 55% of the participants were female, the mean age was 27.2 years (standard deviation = 16.8) and all participants were of Asian origin (Table 2).

Immunogenicity

At D14, every pediatric participant who received PVRV-NG2 (Group 1) and 98% of those who received PVRV (Group 2) achieved RVNA titer ≥0.5 IU/ml; by D42, 100% of participants in Groups 1 and 2 achieved RVNA titer ≥0.5 IU/ml; by D90, 99% of those who received PVRV-NG2 (Group 1) and 98% of those who received PVRV (Group 2) maintained an RVNA titer ≥0.5 IU/ml (Table 3). All pediatric participants had RVNA titer ≥0.2 IU/ml at D14, D42 and D90.

At D14, the proportion of adult participants with RVNA titers ≥0.5 IU/ml was similar between PVRV-NG2 and PVRV groups, ranging from 52 to 75%, but this was lower than that of the pediatric participants (Table 3). By D42, 96 and 100% of participants in Group 5 and 6 respectively, achieved RVNA titer ≥0.5 IU/ml; in Group 5, one participant had an RVNA titer <0.2 IU/ml. By D42, the proportion of adult participants achieving RVNA titers ≥0.2 IU/ml had increased but dropped slightly by D90, but the proportion remained similar between the two groups at each of

Table 1

Population analysis sets for immunogenicity and safety analyses (randomized participants)

Population	Group 1 (n = 112)	Group 2 (n = 56)	Group 3 (n = 26)	Group 4 (n = 14)	Group 5 (n = 129)	Group 6 (n = 65)	Total (n = 402)
FAS, n (%)	112 (100)	56 (100)	26 (100)	14 (100)	129 (100)	65 (100)	402 (100)
FASI, n (%)	100 (89)	53 (95)	24 (92)	13 (93)	112 (87)	56 (86)	358 (89)
PPAS on D14, n (%)	98 (88)	49 (88)	16 (62)	9 (64)	106 (82)	54 (83)	332 (89)
PPAS on D42, n (%)	98 (88)	52 (93)	0 (0)	0 (0)	78 (60)	37 (57)	265 (66)
SafAS, n (%)	112 (100)	56 (100)	26 (100)	14 (100)	129 (100)	65 (100)	402 (100)

Group 1: PVRV-NG2 alone (pediatric); Group 2: PVRV alone (pediatric); Group 3: PVRV-NG2 + ERIG (adult); Group 4: PVRV + ERIG (adult); Group 5: PVRV-NG2 + HRIG (adult); Group 6: PVRV + HRIG (adult)

D: day; ERIG: equine rabies immunoglobulin; FAS: full analysis set; FASI: full analysis set for immunogenicity; HRIG: human rabies immunoglobulin; PPAS: per-protocol analysis set; PVRV: purified Vero rabies vaccine; PVRV-NG2: new generation purified Vero rabies vaccine formulation 2; SafAS: safety analysis set

Table 2

Baseline demographic characteristics of randomized participants (full analysis set)

Characteristic	Group 1 (*n* = 112)	Group 2 (*n* = 56)	Group 3 (*n* = 26)	Group 4 (*n* = 14)	Group 5 (*n* = 129)	Group 6 (*n* = 65)	Total (*n* = 402)
Average age, mean ± SD	10.1 (4.3)	10.8 (3.8)	36.2 (10.4)	37.3 (11.1)	40.2 (11.1)	39.2 (10.9)	27.2 (16.8)
Age group, *n* (%)							
12-23 months	2 (2)	1 (2)	N/A	N/A	N/A	N/A	3 (1)
2-11 years	54 (48)	27 (48)	N/A	N/A	N/A	N/A	81 (20)
12-17 years	56 (50)	28 (50)	N/A	N/A	N/A	N/A	84 (21)
18-40 years	N/A	N/A	15 (58)	8 (57)	65 (50)	38 (58)	126 (31)
41-64 years	N/A	N/A	11 (42)	6 (43)	60 (47)	26 (40)	103 (26)
≥65 years	N/A	N/A	0 (0)	0 (0)	4 (3)	1 (1)	5 (1)
Gender, *n* (%)							
Male	58 (52)	32 (57)	8 (31)	5 (36)	51 (40)	26 (40)	180 (45)
Female	54 (48)	24 (43)	18 (69)	9 (64)	78 (61)	39 (60)	222 (55)

Group 1: PVRV-NG2 alone (pediatric); Group 2: PVRV alone (pediatric); Group 3: PVRV-NG2 + ERIG (adult); Group 4: PVRV + ERIG (adult); Group 5: PVRV-NG2 + HRIG (adult); Group 6: PVRV + HRIG (adult)

ERIG: equine rabies immunoglobulin; HRIG: human rabies immunoglobulin; N/A: not applicable; PVRV: purified Vero rabies vaccine; PVRV-NG2: new generation purified Vero rabies vaccine formulation 2; SD: standard deviation

the two time points. At D90, 75 and 78% of participants in Group 5 and 6 respectively, achieved RVNA titer ≥0.5 IU/ml (Table 3).

At D14, GMTs were higher in pediatric participants (Groups 1 and 2) than those in adult participants (Groups 3-6) (Fig 3A). Overall, GMTs increased from D14 to D42, with values being higher in pediatric participants (Groups 1 and 2) than in adult participants (Groups 5 and 6) (Fig 3B). By D90, GMTs had decreased in pediatric and adult participants to levels similar to those at D14 (Fig 3C).

Safety outcome

No major safety concerns were reported in the pediatric groups (Groups 1 and 2) or in adults with concomitant HRIG administration (Groups 5 and 6) (Table 4). No differences in the major safety concerns were observed between pediatric participants receiving PVRV-NG2 and PVRV as a stand-alone vaccine or in adult participants when administered concomitantly with HRIG.

No immediate unsolicited AEs were reported after any vaccine injection in any group. There was a trend towards a higher proportion (70%) of pediatric participants (Groups 1 and 2) reporting a solicited injection site reaction after any injection compared with adult participants (Group 5, 44%; Group 6, 46%); the most common injection-site reaction was swelling among pediatric participants and pain among adult participants (Table 4). On the other hand, the proportion of participants reporting at least one solicited systemic reaction was higher for adults (Group 5, 52%; Group 6, 49%) than for pediatrics (Group 1, 31%; Group 2, 27%), with headache and myalgia being the most common of these reactions. The majority of solicited reactions were mild to moderate in intensity and occurred within three days of vaccination (Table 4). Overall, the occurrence and the intensity of solicited injection sites and solicited systemic events tended to decrease with incremental number of injections of both vaccines in both age groups (Tables 5 and 6).

During the active phase, six participants (23 % [95 % CI = 9- 44]) in Group 3 and two participants (14% [95% CI: 2-43]) in Group 4 were discontinued from the study

Table 3

RVNA titers using RFFIT method (per-protocol analysis set)

RVNA titer	Group 1	Group 2	Group 3*	Group 4*	Group 5	Group 6
D14, post-dose 3 (PPAS for D14)						
Participants with available RVNA titers at D14 (*n*)	98	49	16	9	106	54
RVNA titer ≥0.2						
n (%)	98 (100)	49 (100)	16 (100)	7 (78)	90 (85)	47 (87)
95% CI	96-100	93-100	79-100	40-97	77-91	75-95
RVNA titer ≥0.5						
n (%)	98 (100)	48 (98)	12 (75)	5 (56)	63 (59)	28 (52)
95 %CI	96-100	89-100	48-93	21-86	49-69	38-66
D42, post-dose 4 (PPAS for D42)						
Participants with available RVNA titers at D42 (*n*)	98	52	N/A	N/A	78	37
RVNA titer ≥0.2						
n (%)	98 (100)	52 (100)	N/A	N/A	77 (99)	37 (100)
95% CI	96-100	93-100	N/A	N/A	93-100	90-100
RVNA titer ≥0.5						
n (%)	98 (100)	52 (100)	N/A	N/A	75 (96)	37 (100)
95% CI	96-100	93-100	N/A	N/A	89-99	90-100

Table 3 (cont)

RVNA titer	Group 1	Group 2	Group 3*	Group 4*	Group 5	Group 6
	D90, post-dose 4 (PPAS for D42)					
Participants with available RVNA titers at D90 (*n*)	97	52	N/A	N/A	76	37
RVNA titer ≥0.2						
n (%)	97 (100)	52 (100)	N/A	N/A	67 (88)	35 (95)
95% CI	96-100	93-100	N/A	N/A	79-94	82-99
RVNA titer ≥0.5						
n (%)	96 (99)	51 (98)	N/A	N/A	57 (75)	29 (78)
95% CI	94-100	90-100	N/A	N/A	64-84	62-90

Group 1: PVRV-NG2 alone (pediatric); Group 2: PVRV alone (pediatric); Group 3: PVRV-NG2 + ERIG (adult); Group 4: PVRV + ERIG (adult); Group 5: PVRV-NG2 + HRIG (adult); Group 6: PVRV + HRIG (adult)

*Groups 3 and 4 were discontinued prior to Dose 4 due to reports of serious adverse events related to ERIG and replaced by Groups 5 and 6.

CI: confidence interval; D: day; ERIG: equine rabies immunoglobulin; HRIG: human rabies immunoglobulin; N/A: not applicable; PPAS: per-protocol analysis set; PVRV: purified Vero rabies vaccine; PVRV-NG2, new generation purified Vero rabies vaccine formulation 2; RFFIT: rapid fluorescent focus inhibition test; RVNA: rabies virus neutralizing antibodies

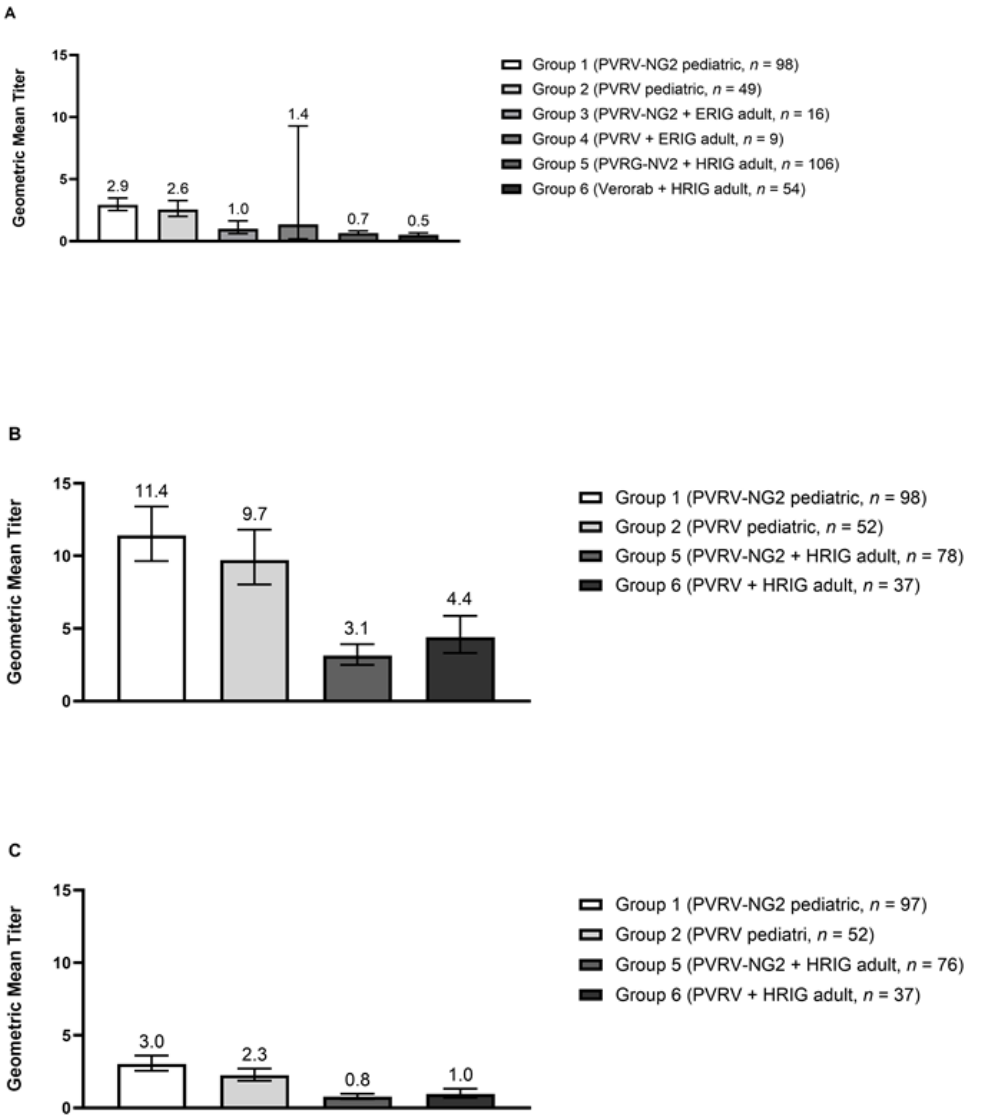

Fig 3 - Geometric mean titers in the per-protocol analysis set (PPAS)

Bar graphs showing geometric mean titers for Groups 1, 2, 5, and 6 at three different time points. A: D14 (PPAS for D14). B: D42 (PPAS for D42). C: D90 (PPAS for D42)

D: day; ERIG: equine rabies immunoglobulin; HRIG: human rabies immunoglobulin; PPAS: per-protocol analysis set; PVRV: purified Vero rabies vaccine; PVRV-NG2: new generation purified Vero rabies vaccine formulation 2

Table 4

Summary of safety outcomes after any vaccination (SafAS)

Safety outcome	Group 1 (n = 112)		Group 2 (n = 56)		Group 3 (n = 26)		Group 4 (n = 14)		Group 5 (n = 129)		Group 6 (n = 65)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Participants experiencing at least one of the following within 30 minutes of any vaccine injection*												
Immediate unsolicited AE	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
Immediate unsolicited AR	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
Participants experiencing at least one of the following within the solicited period after vaccine injection*												
Solicited reaction	84 (75)	66-83	40 (71)	58-83	18 (69)	48-86	8 (57)	29-82	80 (62)	53-70	38 (58)	46-71
Grade 3 solicited reaction	0 (0)	0-3	0 (0)	0-6	2 (8)	1-25	0 (0)	0-2	3 (2)	0-7	1 (2)	0-8
Solicited injection site reaction	78 (70)	60-78	39 (70)	56-81	14 (54)	33-73	5 (36)	13-65	57 (44)	35-53	30 (46)	34-59
Grade 3 injection site reaction	0 (0)	0-3	0 (0)	0-6	1 (4)	0-20	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
Solicited systemic reaction	35 (31)	23-41	15 (27)	16-40	14 (54)	33-73	8 (57)	29-82	67 (52)	43-61	32 (49)	37-62
Grade 3 systemic reaction	0 (0)	0-3	0 (0)	0-6	1 (4)	0-2	0 (0)	0-2	3 (2)	0-7	1 (2)	0-8

Table 4 (cont)

Safety outcome	Group 1 (n = 112)		Group 2 (n = 56)		Group 3 (n = 26)		Group 4 (n = 14)		Group 5 (n = 129)		Group 6 (n = 65)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Up to 28 days after any vaccine injection*												
Unsolicited AE	22 (20)	13-28	12 (21)	12-34	26 (100)	87-100	12 (86)	57-98	4 (3)	1-8	2 (3)	0-11
Unsolicited AR	0 (0)	0-3	0 (0)	0-6	6 (23)	9-44	2 (14)	2-43	0 (0)	0-3	0 (0)	0-5
Unsolicited injections site AR	0 (0)	0-3	0 (0)	0-6	0 (0)	0-13	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
Unsolicited systemic AE	22 (20)	13-28	12 (21)	12-34	26 (100)	87-100	12 (86)	57-98	4 (3)	1-8	2 (3)	0-11
Unsolicited systemic AR	0 (0)	0-3	0 (0)	0-6	6 (23)	9-44	2 (14)	2-43	0 (0)	0-3	0 (0)	0-5
SAE	0 (0)	0-3	0 (0)	0-6	1 (4)	0-20	2 (14)	2-43	1 (1)	0-4	2 (3)	0-11
Death	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
AESI	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
During the vaccination active period†												
AE leading to study discontinuation	0 (0)	0-3	0 (0)	0-6	6 (23)	9-44	2 (14)	2-43	0 (0)	0-3	0 (0)	0-5
SAE	0 (0)	0-3	0 (0)	0-6	1 (4)	0-20	2 (14)	2-43	1 (1)	0-4	2 (3)	0-11
Death	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
AESI	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5

Table 4 (cont)

Safety outcome	Group 1 (n = 112)		Group 2 (n = 56)		Group 3 (n = 26)		Group 4 (n = 14)		Group 5 (n = 129)		Group 6 (n = 65)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
During the 6-month follow-up period[‡]												
SAE	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	4 (3)	1-8	1 (1)	0-8
Death	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
AESI	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
During the study												
SAE	0 (0)	0-3	0 (0)	0-6	1 (4)	0-20	2 (14)	2-43	5 (4)	1-9	3 (5)	1-13
Death	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5
AESI	0 (0)	0-3	0 (0)	0-6	0 (0)	0-1	0 (0)	0-2	0 (0)	0-3	0 (0)	0-5

Group 1: PVRV-NG2 alone (pediatric); Group 2: PVRV alone (pediatric); Group 3: PVRV-NG2 + ERIG (adult); Group 4: PVRV + ERIG (adult); Group 5: PVRV-NG2 + HRIG (adult); Group 6: PVRV + HRIG (adult)

*Related: relationship reported by investigator as related to study vaccine. If the relationship to study vaccine (investigational product) is missing, the event is considered as related. Within solicited period: solicited injection site reactions from Day 0 through Day 7 after each dose; solicited systemic reactions between each dose if doses are separated by less than 7 days, and up to 7 days after each dose if doses are separated by 7 days or more. Up to 28 days: Unsolicited injection site reactions were analyzed up to 28 days after each dose; unsolicited systemic AEs were analyzed between each dose if doses occurred less than 28 days apart, otherwise up to 28 days after each dose.

[†]From date of first exposure to investigational product (Day 0) to 28 days after the date of last exposure to investigational product (date of last exposure + 28 days).

[‡]From 29 days after the date of last exposure to investigational product (date of last exposure + 29 days) to end of 6 months safety follow-up period.

AE: adverse event; AESI: adverse event of special interest; AR: reactions related to study vaccine; CI: confidence interval; ERIG: equine rabies immunoglobulin; HRIG: human rabies immunoglobulin; PVRV: purified Vero rabies vaccine; PVRV-NG2: new generation purified Vero rabies vaccine formulation 2; SAE: serious adverse event; SafAS: safety analysis set

due to at least one AR (Table 4).

No SAEs were reported in pediatric participants (Groups 1 and 2). Overall, 11 SAEs were reported during the study, all among adults, six who received PVRV-NG2 (Groups 3 and 5) and five who received PVRV (Groups 4 and 6) (Table 4). Three of the reported SAEs were considered related to ERIG by the sponsor, namely, one participant who received PVRV-NG2+ERIG (hypersensitivity) and two participants who received PVRV+ERIG (allergic vasculitis and angioedema of lips), which led to discontinuation of ERIG. The remaining SAEs were considered not related to the vaccine or the RIG. No death or AESI was reported during the study.

DISCUSSION

PVRV-NG2 and PVRV displayed comparable immunogenicity patterns and similar safety profiles in pediatric and adult participants. PVRV has been licensed for over thirty years and is currently used in around 80 countries worldwide. WHO also prequalifies PVRV and recommends vaccination through both IM and ID routes (Gongal and Sampath, 2019; Moulenat *et al*, 2020).

All participants received four doses of rabies vaccines, each comprising two 0.1 ml injections administered ID into each arm, as a stand-alone vaccine in healthy pediatric participants or co-administered with RIG at D0 in healthy adults. An updated four-dose TRC intradermal regimen was chosen for this study. Although the current WHO guidelines recommend a three-dose regimen, these are in the process of being adopted or have not yet been adopted in several countries where rabies is endemic (WHO, 2018a); nonetheless, we also evaluated samples seven days after the third dose to allow for informed comparison with the recommended regimen.

This study showed that at D42 (14 days after the fourth dose), almost all participants achieved RVNA titer ≥0.5 IU/ml. GMTs ranged from 3.13 to 11.4 in both age groups and were comparable between PVRV-NG2 and PVRV. These results are in line with the literature, as almost all participants achieved RVNA titers ≥0.5 IU/ml using the ID route with currently licensed vaccines (Preiss *et al*, 2018; Moulenat *et al*, 2020; Xu *et al*, 2021).

Table 5

Solicited injection site reactions up to seven days after each vaccine injection (SafAS)

Solicited injection site reaction	Group 1 (*n* = 112)		Group 2 (*n* = 56)		Group 3 (*n* = 26)		Group 4 (*n* = 14)		Group 5 (*n* = 129)		Group 6 (*n* = 65)	
	n (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI
Injection site tenderness/pain[†]	44 (39)	30-49	23 (41)	28-55	14 (54)	33-73	5 (36)	13-65	57 (44)	35-53	30 (46)	34-59
Post-dose 1	30 (27)	19-36	19 (34)	22-48	13 (50)	30-70	4 (29)	8-58	35 (27)	20-36	20 (31)	20-43
Post-dose 2	33 (29)	21-39	16 (29)	17-42	4 (17)	5-39	1 (7)	0-34	25 (19)	13-27	11 (17)	9-28
Post-dose 3	23 (21)	13-29	6 (11)	4-22	2 (10)	1-30	2 (17)	2-48	31 (24)	17-32	13 (20)	11-32
Post-dose 4	18 (16)	10-24	8 (14)	6-26	N/A	N/A	N/A	N/A	24 (19)	12-27	12 (19)	10-30
Injection site erythema	40 (36)	27-45	15 (27)	16-40	0 (0)	0-13	0 (0)	0-23	0 (0)	0-3	2 (3)	0-11
Post-dose 1	20 (18)	11-26	8 (14)	6-26	0 (0)	0-13	0 (0)	0-23	0 (0)	0-3	0 (0)	0-5
Post-dose 2	22 (20)	13-28	10 (18)	9-30	0 (0)	0-15	0 (0)	0-23	0 (0)	0-3	1 (2)	0-8
Post-dose 3	23 (20)	13-29	11 (20)	10-32.	0 (0)	0-16	0 (0)	0-26	0 (0)	0-3	0 (0)	0-5
Post-dose 4	31 (28)	20-37	12 (21)	12-34	N/A	N/A	N/A	N/A	0 (0)	0-3	1 (2)	0-8

Table 5 (cont)

Solicited injection site reaction	Group 1 (*n* = 112)		Group 2 (*n* = 56)		Group 3 (*n* = 26)		Group 4 (*n* = 14)		Group 5 (*n* = 129)		Group 6 (*n* = 65)	
	n (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI
Injection site swelling	53 (47)	38-57	27 (48)	35-62	0 (0)	0-13	0 (0)	0-23	0 (0)	0-3	0 (0)	0-5
Post-dose 1	28 (25)	17-34	10 (18)	9-30	0 (0)	0-13	0 (0)	0-23	0 (0)	0-3	0 (0)	0-5
Post-dose 2	32 (29)	20-38	18 (32)	20-46	0 (0)	0-15	0 (0)	0-23	0 (0)	0-3	0 (0)	0-5
Post-dose 3	41 (37)	28-46	20 (36)	23-50	0 (0)	0-16	0 (0)	0-26	0 (0)	0-3	0 (0)	0-5
Post-dose 4	46 (41)	32-51	21 (37)	25-51	N/A	N/A	N/A	N/A	0 (0)	0-3	0 (0)	0-5

Group 1: PVRV-NG2 alone (pediatric); Group 2: PVRV alone (pediatric); Group 3: PVRV-NG2 + ERIG (adult); Group 4: PVRV + ERIG (adult); Group 5: PVRV-NG2 + HRIG (adult); Group 6: PVRV + HRIG (adult)

*Groups 3 and 4 were discontinued prior to Dose 4 due to reports of serious adverse events related to ERIG and replaced by Groups 5 and 6.

†Injection site tenderness for participants 2-23 months of age; injection site pain for participants ≥2 years of age

CI: confidence interval; ERIG: equine rabies immunoglobulin; HRIG: human rabies immunoglobulin; N/A: not applicable; SafAS: safety analysis set

Table 6

Solicited systemic reactions up to seven days after each vaccine injection (SafAS)

Solicited systemic reaction*	Group 1 (n = 112)		Group 2 (n = 56)		Group 3 (n = 26)		Group 4 (n = 14)		Group 5 (n = 129)		Group 6 (n = 65)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Fever	1 (1)	0-5	0 (0)	0-6	2 (8)	1-25	0 (0)	0-23	2 (2)	0-5	2 (3)	0-11
Post-dose 1	0 (0)	0-3	0 (0)	0-6	0 (0)	0-13	0 (0)	0-23	0 (0)	0-3	0 (0)	0-5
Post-dose 2	1 (1)	0-5	0 (0)	0-6	0 (0)	0-15	0 (0)	0-23	0 (0)	0-3	0 (0)	0-5
Post-dose 3	0 (0)	0-3	0 (0)	0-6	2 (10)	1-30	0 (0)	0-23	0 (0)	0-3	0 (0)	0-5
Post-dose 4	0 (0)	0-3	0 (0)	0-6	N/A	N/A	N/A	N/A	2 (2)	0-6	2 (3)	0-11
Headache	20 (18)	11-27	10 (18)	9-31	5 (19)	7-39	3 (21)	5-51	31 (24)	17-32	11 (17)	9-28
Post-dose 1	11 (10)	5-17	7 (13)	5-24	4 (15)	4-35	1 (7)	0-34	19 (15)	9-22	7 (11)	4-21
Post-dose 2	8 (7)	3-14	3 (5)	1-15	3 (13)	3-34	2 (14)	2-43	10 (8)	4-14	2 (3)	0-11
Post-dose 3	7 (6)	3-13	4 (7)	2-18	0 (0)	0-16	2 (17)	2-48	10 (8)	4-14	3 (5)	1-13
Post-dose 4	6 (5)	2-11	1 (2)	0-10	N/A	N/A	N/A	N/A	8 (6)	3-12	2 (3)	0-11
Malaise	18 (16)	10-25	9 (16)	8-29	6 (23)	9-44	5 (36)	13-65	39 (30)	22-39	13 (20)	11-32
Post-dose 1	13 (12)	6-19	6 (11)	4-22	5 (19)	7-39	4 (29)	8-58	26 (20)	14-28	5 (8)	2-17
Post-dose 2	8 (7)	3-14	4 (7)	2-18	2 (9)	1-28	1 (7)	0-34	17 (13)	8-20	3 (5)	1-13
Post-dose 3	3 (3)	1-8	2 (4)	0-12	1 (5)	0-24	1 (8)	0-38	17 (13)	8-20	5 (8)	2-17
Post-dose 4	6 (5)	2-11	2 (4)	0-12	N/A	N/A	N/A	N/A	12 (9)	5-16	5 (8)	2-17

Table 6 (cont)

Solicited systemic reaction*	Group 1 (*n* = 112)		Group 2 (*n* = 56)		Group 3 (*n* = 26)		Group 4 (*n* = 14)		Group 5 (*n* = 129)		Group 6 (*n* = 65)	
	n (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI
Myalgia	26 (24)	16-33	10 (18)	9-31	11 (43)	23-63	8 (57)	29-82	61 (47)	38-56	25 (38)	27-51
Post-dose 1	13 (12)	6-19	6 (11)	4-22	11 (42)	23-63	6 (43)	18-71	44 (34)	26-43	21 (32)	21-45
Post-dose 2	16 (15)	9-23	6 (11)	4-22	3 (13)	3-34	0 (0)	0-23	25 (19)	13-27	11 (17)	9-28
Post-dose 3	5 (5)	1-10	4 (7)	2-18	1 (5)	0-24	2 (17)	2-48	21 (16)	10-24	8 (12)	5-23
Post-dose 4	7 (6)	3-13	4 (7)	2.-18	N/A	N/A	N/A	N/A	20 (16)	10-23	6 (9)	3-19

Group 1: PVRV-NG2 alone (pediatric); Group 2: PVRV alone (pediatric); Group 3: PVRV-NG2 + ERIG (adult); Group 4: PVRV + ERIG (adult); Group 5: PVRV-NG2 + HRIG (adult); Group 6: PVRV + HRIG (adult)

*Solicited systemic reaction data collected differed by age group. Headache, malaise, and myalgia were collected for participants ≥ 2 years of age in all groups. Vomiting, abnormal crying, drowsiness, appetite lost, and irritability were collected only for participants 12-23 months of age (n = 2 in Group 1 and n = 1 in Group 2). In Group 1, abnormal crying was reported in 1 participant (50 %) after each dose and irritability was reported in 1 participant (50 %) post-dose 4, whereas vomiting, drowsiness, and appetite lost were not reported in any participants; none of these events were reported in the participant in Group 2.

†Groups 3 and 4 were discontinued prior to Dose 4 due to reports of serious adverse events related to ERIG and replaced by Groups 5 and 6.

CI: confidence interval; ERIG: equine rabies immunoglobulin; HRIG: human rabies immunoglobulin; N/A: not applicable; PPAS: per-protocol analysis set; PVRV: purified Vero rabies vaccine; PVRV-NG2: new generation purified Vero rabies vaccine formulation 2; SafAS: safety analysis set

By D14 (seven days after the third dose), the proportion of adult participants with RVNA titers ≥0.2 IU/ml ranged from 85% (Group 5) to 87% (Group 6), indicating the development of an immune response. However, approximately half of all adult participants (Group 5, 59%; Group 6, 52%) achieved RVNA titers ≥0.5 IU/ml. As such, the immunogenicity observed in adult participants at D14 after three doses of either vaccine was lower than the historical data using the ID route regarding the proportion of participants achieving RVNA titers ≥0.5 IU/ml (Morelli *et al*, 2022). Notably, almost all adult participants achieved RVNA titers ≥0.5 IU/ml by D42 (Group 5, 96%; Group 6, 100%). Considering the extensive data available on ID administration of PVRV in adult participants, the immunogenicity results at D14 observed in the current study were unexpected and have not been observed elsewhere (Moulenat *et al*, 2020; Auerswald *et al*, 2023). In contrast, nearly all pediatric participants achieved RVNA titers ≥0.5 IU/ml by D14, with GMTs increasing from baseline through D42. These results are consistent with those in the literature, which describes a rapid immunological response in the pediatric population (Xu *et al*, 2021).

To better understand the abovementioned results, extensive investigations were conducted into study sites, products and laboratory procedures; however, no root cause for the unexpected results could be identified. At the site level, there were no deviations from the operating procedures and no issues with product quality or laboratory/sample procedures. WHO has previously emphasized the importance of a correct ID technique, which should result in a wheal/papule at the injection site (Dreesen *et al*, 1984; WHO, 2018b); however, the potential for incorrect ID administration at one particular study site could not be excluded due to the absence of documentation describing the observation of the wheal/papule 30 minutes post-exposure (Papania *et al*, 2018). Although the personnel who performed the ID injections were trained before the study, they cannot be considered ID vaccination experts. Furthermore, all adult participants with RVNA

titers ≤0.5 IU/ml at D14 were vaccinated at this single study site, and all participants received concomitant administration of either ERIG or HRIG. To ensure that the correct procedure is followed, future investigations should document the observation of the wheal/papule following ID vaccine administration.

Numerous clinical effectiveness studies and large post-marketing experience support ID PVRV PEP, regardless of schedule, with almost all participants achieving a sufficient immune response at D14 (Moulenat *et al*, 2020). For example, a previous study investigating PVRV found that a three-dose ID IPC PEP regimen is as effective at inducing a high immune response as a four-dose ID Essen PEP regimen, with almost all participants achieving sufficient neutralizing antibody titers (≥0.5 IU/ml) by D14 (Auerswald *et al*, 2023). A systematic review also found that ID PVRV PEP induces a rapid and persistent immune response, regardless of the regimen used (Moulenat *et al*, 2020). It is important to note that in the current study immune responses were similar between PVRV and PVRV-NG2. In keeping with the trend reported here, data generated through multiple clinical trials assessing IM administration also support the immunogenicity and favorable safety profile of PVRV-NG2 (Chansinghakul *et al*, 2024; Pineda-Peña *et al*, 2024). Of note, during the current study, the IPC regimen was evaluated at D14 (7 days after the third vaccination), with no further time points to assess the kinetics of the immune response over time, as all study participants received a fourth dose at D28.

A simulated PEP regimen is the most stringent evaluation of a rabies vaccine (WHO, 2018b; Morelli *et al*, 2022; Schreuder *et al*, 2020), and the potential for enhanced interference of RIG in a simulated PEP setting should be noted as a contributing factor to the unexpected results among the adult participants. In this simulated PEP setting, the full RIG dose was injected IM into the participants' thighs; whereas according to the WHO guidelines, RIGs should be infiltrated in and around the wound in the event of exposure to a suspect rabid animal

(WHO, 2018a). Interference of RIG with the vaccine is a well-known phenomenon for both IM and ID routes of administration (Loofbourow *et al*, 1971; Matson *et al*, 2020; Bookstaver *et al*, 2021). RVNA titers are often lower in those receiving HRIG or ERIG compared with those receiving the vaccine alone (Preiss *et al*, 2018; Quiambao *et al*, 2020). Despite its known interference with rabies vaccinations, RIG for Category III wounds remains essential to cover the early post-exposure period (Khawplod *et al*, 2002; WHO, 2018a).

In the current study, the immunogenicity results at D90 supported the persistence of the immune response, whereby >98 % of the pediatric population still showed RVNA titers ≥0.5 IU/ml, which is consistent with the literature (Quiambao *et al*, 2019). On the other hand, a lower proportion of the adult participants showed RVNA titers ≥0.5 IU/ml for both groups at D90 (75% for PVRV-NG2+HRIG at D0 and 78% for PVRV+HRIG at D0) when compared with PVRV historical data (Dreesen, 1997; Toovey, 2007; Pichon *et al*, 2023). These results may be related to the low immune response observed in this age group at D14 and the possibility that ID administration was suboptimal.

PVRV-NG2 was well tolerated and displayed a comparable safety profile to that of PVRV. In addition, both vaccines were well tolerated in adults who received concomitant HRIG administration (Groups 5 and 6). PVRV has been licensed for decades and listed as a WHO-prequalified rabies vaccine since 2005 (Toovey, 2007), with a well-established satisfactory safety profile. As PVRV-NG2 is a purified version of PVRV, developed using the same Wistar Rabies Pitman Moore/WI 38 1503-3M strain, and is pharmaceutically comparable to PVRV, it was expected to show a similar safety profile to PVRV. The satisfactory safety profile of PVRV-NG2 observed in the current study was also consistent with previous studies using the IM route of administration (Chansinghakul *et al*, 2024; Pineda-Peña *et al*, 2024).

As previously mentioned, the results observed in the current study with the immunogenicity of PVRV-NG2 via the IM route were unexpected but isolated in

comparison with other studies (Chansinghakul *et al*, 2024; Pineda-Peña *et al*, 2024). Despite extensive investigations into the unexpected results, no single cause was identified and is likely multifactorial. Further investigation is required to better understand the immunological response through administration via the ID route with concomitant RIG.

In conclusion, PVRV-NG2 and PVRV showed comparable immunogenicity and equally favorable safety profiles after ID administration, either alone or concomitantly with HRIG, in a PEP regimen at all time points. By D14, after three vaccinations, PVRV-NG2 elicited a favorable immune response among the pediatric participants, whereas a lower immune response was observed in adults who received concomitant HRIG. However, by D28, after four vaccinations via the ID route, all age groups demonstrated an adequate immune response. Overall, PVRV-NG2 was well tolerated in both pediatric and adult participants, with no safety concerns identified.

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CONFLICT OF INTEREST DISCLOSURE

Danaya Chansinghakul, Carina Frago, Qian Jiang, Andrea-Clemencia Pineda-Peña, Celine Petit, Elisa Valero, and Manuel Vangelisti are employees of Sanofi and may hold shares and/or stock options in the company.

Kriengsak Limkittikul and Piroon Mootsikapun declare no conflicts of interest.

DATA AVAILABILITY

Qualified researchers may request access to patient-level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient-level data will be anonymized, and study documents will be redacted to protect the privacy of study participants. Further details regarding Sanofi data-sharing criteria, eligible studies and process for requesting access can be found at https://www.vivli.org/.

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