A phase II randomized study to determine the safety and immunogenicity of the novel PIKA rabies vaccine containing the PIKA adjuvant using an accelerated regimen

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ABSTRACT

Background: Human Rabies infection continues to be potentially fatal despite the availability of post-exposure prophylaxis with rabies vaccine. The PIKA Rabies vaccine adjuvant is a TLR3 agonist and has been shown to be safe and immunogenic in clinical phase I studies.

Methods: We conducted a phase II, open label, randomized study in healthy adults to assess the safety and immunogenicity of the PIKA rabies vaccine under an accelerated regimen. 126 subjects were randomly assigned into two groups: control vaccine classic regimen ("control-classic") and PIKA vaccine accelerated regimen ("PIKA-accelerated"). Subjects were followed up for safety and rabies virus neutralizing antibodies (RVNA).

Results: Both the control and PIKA vaccines were generally well tolerated. 57.6% of subjects in the PIKA vaccine group, compared with 43.8% of subjects in the control-classic group, achieved the target RVNA titer of ≥0.5 IU/mL by Day 7. All subjects achieved the target RVNA titer by Day 14. The RVNA geometric mean titer at Day 7 was 0.60 IU/ml in the PIKA vaccine group and 0.39 IU/ml in the control-classic group. At Day 14, the RVNA geometric mean titer was 18.25 IU/ml in the PIKA-accelerated group and 19.24 IU/ml in the control-classic group. The median time taken to reach the target RVNA titer level of ≥0.5 IU/mL was 7.0 days (95% CI: 7.0–42.0 days) in the PIKA-accelerated group and 14.0 days (95% CI: 7.0–42.0 days) in the control-classic group.

Conclusion: The accelerated regimen using the investigational PIKA Rabies vaccine was well-tolerated and demonstrated non-inferior immunogenicity compared to the classic regimen using the commercially available vaccine in healthy adults.

Clinical trial registry: The study was registered with clinicaltrials.gov (NCT02956421).

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Abbreviations: ACIP, Advisory Committee on Immunization Practices; AE, adverse event; CI, confidence interval; CIRB, Centralised Institutional Review Board; CSIRO, Commonwealth Scientific and Industrial Research Organization; CTCAE, Common Terminology Criteria for Adverse Events; CTRU, Clinical Trials Research Unit; cCRF, electronic case record form; FAVN, fluorescent antibody virus neutralization; GMP, Good Manufacturing Practice; GMT, geometric mean titer; HSA, Health Sciences Authority; IMU, Investigational Medicine Unit; IPRV, Inactivated and Purified Rabies Virus; MEdDRA, Medical Dictionary for Regulatory Activities; PEP, post-exposure prophylaxis; PIKA, Polyinosinic-Polycytidylic Acid Based Adjuvant; RFFIT, rabies fluorescent focus inhibition test; RIG, rabies immunoglobulin; RVNA, rabies virus neutralizing antibody; SAE, serious adverse event; TLR3, toll-like receptor 3; WHO, World Health Organization.

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1. Background

Human Rabies is entirely preventable, yet remains one of the most neglected infectious diseases with tens of thousands of deaths annually. The majority of cases occur in Asia, with 40% of cases occurring in children. Rabies is almost always fatal, but can be prevented by immediate local wound treatment and post-exposure prophylaxis (PEP) [1], as well as co-administration of rabies immunoglobulin (RIG) in appropriate cases [2]. The two most common PEP regimens are the abbreviated Essen (four injections administered on days 0, 3, 7, 14) [3] and the Zagreb schedule (two injections administered on day zero and one injection on days 7 and 21 each) [4]. PEP needs to be administered as early as possible to give the best chance of protection against developing clinical disease.

Fatalities however, are not uncommon even after administration of appropriate PEP, particularly in cases where RIG is either not been administered or has been administered inappropriately [5,6]. The short incubation period of the disease results in insufficient time for the development of a protective immune response [5,6]. As such, a shorter, accelerated and cost saving regimen is therefore appealing as it could enhance vaccination compliance, and may allow for earlier development of adaptive immunity, which could improve survival post-exposure. This is of pertinence particularly in developing countries where RIG is extremely expensive and not easily available, often making PEP the sole means of protection.

The PIKA adjuvant is a synthetic analogue of a dsRNA and a refined form of Polyinosinic-Polycytidylic Acid stabilized with kanamycin and calcium [7–9]. It is a TLR3 agonist capable of activating potent antigen presenting cells such as dendritic cells leading to a more robust adaptive immunity [8,9]. Animal studies have shown that administration of the PIKA rabies vaccine using an accelerated schedule results in increased survival in infected hamsters [10] and high titers of IgG and IgM production as early as four days after immunization [11].

The results of a recently completed Phase I clinical trial conducted by our group demonstrated the safety and immunogenicity of the PIKA rabies vaccine compared with the control vaccine (Novartis RABIPUR®) [12]. Of note, we showed that with an accelerated regimen (2-2-1 schedule of a double dose injection on Days 0 and 3, and a single dose injection on Day 7), the PIKA rabies vaccine had a higher protection rate with 75% of subjects reaching an RVNA titer ≥0.5 IU/mL by day 7 post-vaccination compared to 53.9% in the classic PIKA rabies vaccine regimen (1-1-1 schedule of a single dose injection on Days 0, 3, 7 and 14) and only 16.7% in the control vaccine classic regimen, demonstrating the increased immunogenicity of the PIKA-adjuvanted vaccine. In addition, the PIKA rabies vaccine elicited a multi-specific rabies CD4 mediated T-cell response detectable as early at Day 7 that was maintained at Day 42. In view of these promising results, we conducted a phase II non-inferiority study to further investigate the safety and immunogenicity profile of the investigational PIKA rabies vaccine under the accelerated regimen in healthy adults.

2. Material and methods: study design

This was a phase II, multicenter, open-label, randomized study conducted in healthy adults, aged 21–65 years, with no prior history of rabies vaccination. It was conducted at two centers in Singapore: the SingHealth Investigational Medicine Unit (IMU), and the Clinical Trials & Research Unit (CTRU) at Changi General Hospital. The study was designed to evaluate the safety and immunogenicity of the PIKA rabies vaccine administered in an accelerated regimen, against a commercially available rabies vaccine (Novartis RABIPUR®) administered in a classic regimen. Study approval was obtained from the Singapore Health Sciences Authority (HSA CTC1600017 and CTC1600018) and Centralized Institutional Review Board (CIRB Ref: 2015/3061). The study was performed in agreement with the International Conference on Harmonization guidelines on Good Clinical Practices, laws and regulatory requirements in Singapore. The study was registered with clinicaltrials.gov (NCT02956421). Informed consent was obtained from each subject prior to screening. Subjects were first enrolled on 22 February 2016 with the last subject visit on 27 July 2016.

It was planned to recruit approximately 120 subjects in total in order to have at least 48 subjects in each treatment arm reaching the primary end point, based on an estimated 20% dropout rate. In total, 182 subjects were screened, with 126 subjects eventually randomized into two groups (control-classic and PIKA-accelerated) in a 1:1 ratio, using numbers randomly generated by an interactive web-based response system. Subject disposition in the study is illustrated in Fig. 1.

The control-classic group received Rabipur®, a commercially available rabies vaccine (Novartis RABIPUR®, Batch number: 5930111A) containing 7.4 IU/vial of inactivated rabies vaccine. The PIKA-accelerated group received the investigational PIKA rabies vaccine containing 2.0 IU/vial of inactivated rabies vaccine. The control-classic group was vaccinated using the classic 4-dose regimen (1-1-1-1 schedule on Days 0, 3, 7 and 14), whilst the PIKA-accelerated group received an accelerated regimen (2-2-1 schedule with a double-dose injection in Days 0 and 3 and a single-dose injection on Day 7) (Fig. 2). The primary endpoints were safety and immunogenicity induced by the PIKA vaccine. All adverse events (AEs) were captured up to Day 42. The primary immunogenicity endpoint was the proportion of subjects with an RVNA titer of ≥0.5 IU/ml at Day 14. The secondary immunogenicity endpoint was the proportion of subjects with an RVNA titer of ≥0.5 IU/ml at Day 7. An exploratory analysis was also performed by evaluating the RVNA geometric mean titer (GMT) value at Day 0, 7, 14, and 42 for both treatment groups, as well as the time to reach target RVNA titer level of 0.5 IU/ml.

2.1. Subjects and study procedures

Participants 21–65 years of age, with satisfactory baseline medical assessment and laboratory values within the normal ranges were eligible. The full list of inclusion and exclusion criteria is shown in Supplementary Table 1.

All clinical data collection was conducted using an electronic case report form (eCRF) and maintained on a secure web-based database managed by CMIC Asia-Pacific Pte. Ltd, a commercial clinical research organization. Investigators had no access to the database until trial completion.

2.2. Vaccine production

The investigational PIKA rabies vaccine was manufactured by Liaoning Yisheng Bio-Pharmaceutical Co. Ltd in China. These facilities meet GMP standards for quality assurance and control. The CTN-1 rabies virus strain was originally isolated from the brain tissue of a fatal human rabies case in Shandong, China in 1956 (named CTN-1S). After continuous passage in mouse brain cells, it was eventually passaged in Vero cell line (or Vero cells) [13,14].

The vaccine virus solution was first concentrated, and then inactivated by the addition of β-propiolactone solution (with a ratio of β-propiolactone to virus solution of 1:4000). It was mixed evenly for 30 min, stored at a temperature of 4 °C for 24 h and then hydrolyzed by shaking for two hours at 37 °C. After adjusting the pH to 7.6–7.8, the inactivated virus solution was stored at 2–8 °C. Quality control testing performed before the concentration and
inactivation process showed that the viral titer was between 6–8 Ig LD50/ml in 19 sub-batches of the batch II-20130901 that was used for this trial.

The investigational PIKA rabies vaccine is a combination of the Inactivated and Purified Rabies Virus (IPRV) and the PIKA adjuvant combined in a phosphate buffer solution. The amount of antigen in the IPRV is determined using ELISA, as per the standards set by the Chinese Pharmacopoeia 2010 [15]. Based on these results, the designated amount of IPRV and PIKA are mixed to make the final concentration of the semi-finished product at 2.0 IU/ml of inactivated purified rabies antigen and 1.0 mg/ml of PIKA adjuvant. This ratio of antigen and adjuvant mix has been optimized in pre-clinical testing to reduce the dose without compromising its potency. The final product is in freeze-dried form by adding human albumin as a stabilizer and other excipients, namely maltose and dextran. The formulation of one vial of the freeze-dried PIKA rabies vaccine from a standard volume (1 ml) of the vaccine comprises 2 IU of inactivated purified rabies antigen; 1 mg of PIKA adjuvant; 3 µg of human albumin; 30 µg of maltose and 50 µg of dextran. Stability testing of the PIKA rabies vaccine has shown that it remains stable at 37 °C for at least six months, even after 39 months of storage at 2–8 °C.

### 2.3. Safety assessments

Monitoring for AEs included a medical assessment within 2 h prior and post vaccination. Systemic and local injection site AEs were documented up to 2 h after each injection. All subjects were provided with a patient diary to record all solicited and unsolicited AEs up to Day 42. Pre-specified blood tests and urine analyses were taken at all study visits (Supplementary Table 2).

All AEs were classified according to the Medical Dictionary for Regulatory Activities (MedDRA), version 18 and coded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.

### 2.4. Analysis of rabies virus neutralizing antibody (RVNA) production

The endpoint for assessing the humoral response to vaccination was the RVNA titer at Day 7, 14 and 42 after the first injection. RVNA titer levels were batch-tested at the Commonwealth Scientific and Industrial Research Organization (CSIRO)-Australian Animal Health Laboratory at the end of the last subject visit. The fluorescent antibody virus neutralization (FAVN) assay was used to determine the RVNA titer levels [16]. As per WHO requirements,
a titer of 0.5 IU/ml was used as the cut-off value for meeting protection.

2.5. Statistical methods

Immunogenicity was evaluated using a test for non-inferiority based on a two-sided 95% confidence interval (CI) for the difference in the proportion of subjects with RVNA titer >0.5 IU/ml at Day 14 between the PIKA-accelerated and control-classic groups. Non-inferiority was concluded if the lower limit of the two-sided 95% confidence interval was greater than −0.10.

The frequency and percentage of subjects with RVNA titer >0.5 IU/ml as well as descriptive summaries of titer level of RVNA, RVNA GMT and change from baseline were presented for each visit. The proportion of subjects who reached the target RVNA titer level of 0.5 IU/ml at each visit was summarized using Kaplan-Meier survival analysis with a 95% CI. Safety was evaluated by collecting AEs, safety laboratory parameters and humoral immune response data.

3. Results

3.1. Demographic characteristics and enrollment

A total of 182 subjects were screened of whom 126 were randomized. 64 subjects were randomized to the control-classic group and 62 to the PIKA-accelerated group. All subjects in the control-classic group and 59 subjects in the PIKA-accelerated group received the assigned treatment – data from these 123 subjects were included in the full analysis set (FAS), per-protocol set and safety set. 118 subjects completed the study (61 in the control-classic group and 57 in the PIKA-accelerated group). Eight discontinued the study (3 in the control-classic group and 5 in the PIKA-accelerated group). The reasons for study discontinuation are shown in Fig. 1.

There were no significant differences in the baseline demographics of the subjects in each group as shown in Table 1. The mean age of subjects in the control-classic and PIKA groups was 35.2 and 34.1 years, respectively, with a predominance of males. Most of the subjects were Chinese (Control-classic: 78.1% and PIKA-accelerated: 71.2%), as is consistent with the ethnic distribution in Singapore.

3.2. Vaccine safety

A total of 93 subjects reported 335 AEs over the course of the study (Table 2). Most of the AEs reported were mild or moderate in severity. Severe AEs were reported in 4.7% of subjects in the control-classic group versus 6.8% in the PIKA-accelerated group. Only one serious adverse event (SAE) was reported – this occurred in the control-classic group. There were no deaths. Three (4.7%) subjects in the control-classic group and none in the PIKA-accelerated group experienced AEs leading to study discontinuation. One subject was withdrawn due to fever after an acute viral infection six days after the third dose – his fever resolved after 48 h. The last subject had an elevated alanine aminotransferase (Grade 3) post-third dose, which was classified as "probably related" to the control vaccine. This resolved after one week.

The most common systemic AEs reported were fatigue (9.4% [Control-classic] vs. 20.3% [PIKA-accelerated]) followed by pyrexia (6.3% [Control-classic] vs. 6.8% [PIKA-accelerated]), headache (9.4% [Control-classic] vs. 6.8% [PIKA-accelerated]), and induration (9.4% [Control-classic] vs. 13.6% [PIKA-accelerated]). All systemic AEs were graded as mild or moderate. Hyperkalemia was deemed unrelated to vaccination in all subjects except for one subject in the control vaccine before the study team was notified of the tuberculosis diagnosis. The second subject was withdrawn due to fever from an acute pulmonary tuberculosis. This SAE was unrelated to the control vaccine. This resolved after one week.

3.3. Vaccine immunogenicity

By Day 7, 57.6% of subjects in the PIKA-accelerated group and 43.8% in the control-classic group achieved an RVNA titer level of at least 0.5 IU/ml, although this difference did not reach statistical significance (Table 3). The median RVNA titer levels at
Day 7 was 0.42 IU/ml in the control-classic group and 0.66 IU/ml in the PIKA-accelerated group (Table 4a).

By Day 14, all subjects in both groups reached the target RVNA titer level of ≥0.5 IU/ml. The Kaplan-Meier estimated proportions of subjects with RVNA titer level ≥0.5 IU/ml were almost equal in both groups at Day 14 (98.4% [control] vs. 98.3% [PIKA]) and the lower limit of the two-sided 95% CI for the difference was 0, demonstrating non-inferiority of the accelerated regimen using the PIKA rabies vaccine. The median RVNA titer levels at Day 14 was 23.38 IU/ml in both groups (Table 4a).

In both groups, the RVNA GMT increased from baseline at Day 7 and Day 14 (Table 4b). Compared to baseline values, GMT values in both groups were higher at Day 7 (Control: 0.39 IU/ml; PIKA: 0.60 IU/ml) and at Day 14 (Control: 19.24 IU/ml; PIKA: 18.25 IU/ml). The median time taken to reach the target RVNA titer level of ≥0.5 IU/ml was 14.0 days (95% CI: 7.0–42.0 days) in the control-classic group and 7.0 days (95% CI: 7.0–42.0 days) in the PIKA-accelerated group (Table 4c).

4. Discussion

Although rabies is a vaccine preventable disease, mortality due to rabies infections still remains unacceptably high. As such, there remains an urgent unmet need for a more cost effective and efficacious vaccine regimen which is able to produce a robust protective immune response more rapidly than the current standard of care.

In this study, all subjects in both groups achieved the target RVNA titer level of ≥0.5 IU/ml by Day 14. Kaplan-Meier analysis showed that the estimated proportions of subjects achieving an RVNA titer level of ≥0.5 IU/ml by Day 14 were similar in the two groups, demonstrating non-inferiority of the PIKA rabies vaccine under the accelerated regimen compared to the control vaccine under the standard regimen.

At Day 7, the target RVNA titer level ≥0.5 IU/ml was achieved by 57.6% of subjects in the PIKA-accelerated group compared to 43.8% in the control-classic group. Although this difference did not reach statistical significance, this finding is of clinical significance as rapid induction of protective RVNA is critical for effective post-exposure protection.

Results from this study provide further evidence on the immunogenicity and safety of the PIKA rabies vaccine and are largely consistent with those from the Phase I study. As with the Phase I study, all subjects in the PIKA-accelerated and control-classic groups achieved an RVNA titer level of ≥0.5 IU/ml by Day 14. However, despite subject demographics being broadly similar, the proportion of subjects in the control-classic group achieving an RVNA titer level of ≥0.5 IU/ml at Day 7 was somewhat higher in this study than in the Phase I study [12]. This could potentially be explained by the fact that the control vaccine (Novartis RABIPUR®) used in the Phase I study contained only 5.9 IU/dose, compared to a higher antigenic value of 7.4 IU/dose in the control vaccine used in this study. In addition, in the accelerated regimen where two doses of PIKA rabies vaccine were injected simultaneously with doubling of the rabies antigen dose (8.0 IU from the Day 1 and 3 injections), this would still be much lower than the antigen dose provided by the control vaccine (14.8 IU for two doses). The better immunological performance may indeed be due to the higher IU present in doubling the dose of the PIKA vaccine in the accelerated regimen.

### Table 3
Proportion of subjects reaching ≥0.5 IU/ml RVNA.

<table>
<thead>
<tr>
<th>Study day</th>
<th>Control-classic (N = 64)</th>
<th>PIKA-accelerated (N = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Kaplan-Meir estimate of proportion (95% CI)</td>
</tr>
<tr>
<td>Day 7</td>
<td>28 (43.8)</td>
<td>43.8% (32.7, 56.7)</td>
</tr>
<tr>
<td>Day 14</td>
<td>63 (100.0)</td>
<td>98.4% (92.6, 99.9)</td>
</tr>
</tbody>
</table>

Note: Control-classic = Novartis Rabipur® vaccine; PIKA-accelerated = PIKA adjuvant rabies vaccine accelerated regimen.

### Table 4a
Summary of RVNA titer level.

<table>
<thead>
<tr>
<th>RVNA titer, IU/ml</th>
<th>Control-classic (N = 64)</th>
<th>PIKA-accelerated (N = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.11 (0.08)</td>
<td>0.22 (0.76)</td>
</tr>
<tr>
<td>Median</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Range</td>
<td>0.01–0.50</td>
<td>0.06–5.92</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.77 (1.73)</td>
<td>3.59 (20.72)</td>
</tr>
<tr>
<td>Median</td>
<td>0.42</td>
<td>0.66</td>
</tr>
<tr>
<td>Range</td>
<td>0.06–13.50</td>
<td>0.06–159.90</td>
</tr>
<tr>
<td>Day 14 (±2 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>26.50 (19.95)</td>
<td>27.99 (31.71)</td>
</tr>
<tr>
<td>Median</td>
<td>23.38</td>
<td>23.38</td>
</tr>
<tr>
<td>Range</td>
<td>1.25–92.32</td>
<td>1.50–230.62</td>
</tr>
</tbody>
</table>

### Table 4b
RVNA geometric mean titer.

<table>
<thead>
<tr>
<th>GV, IU/ml</th>
<th>Control-classic (N = 64)</th>
<th>PIKA-accelerated (N = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed value</td>
<td>Change from baseline</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>0.39</td>
<td>0.34</td>
</tr>
<tr>
<td>Day 14</td>
<td>19.24</td>
<td>18.99</td>
</tr>
<tr>
<td>Day 42</td>
<td>15.18</td>
<td>15.02</td>
</tr>
</tbody>
</table>

* GMT is calculated by applying a natural-log transformation to each data value, calculating the average of the log-transformed values and finally applying an anti-log transformation to the average value. Negative or zero values may result when calculating change from baseline (e.g. at Day 7 when the numerical change was may be small). Log transformation cannot be applied to these values, which will therefore be excluded from the GMT calculation. Calculated change in GMT may therefore be numerically larger than the GMT calculated from the observed Day 7 values.

### Table 4c
Time to reach target RVNA titer level ≥0.5 IU/ml.

<table>
<thead>
<tr>
<th>RVNA geometric mean titer.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-classic (N = 64)</td>
</tr>
<tr>
<td>PIKA-accelerated (N = 59)</td>
</tr>
<tr>
<td>Mean (SD), days</td>
</tr>
<tr>
<td>11.4 (5.2)</td>
</tr>
<tr>
<td>(95% CI of mean)</td>
</tr>
<tr>
<td>(10.1, 12.7)</td>
</tr>
<tr>
<td>Median, days (95% CI of median)</td>
</tr>
<tr>
<td>14.0 (7.0–42.0)</td>
</tr>
<tr>
<td>7.0 (7.0–42.0)</td>
</tr>
</tbody>
</table>

Note: All values are in IU/ml unless otherwise stated; Control-classic = Novartis Rabipur® vaccine; PIKA-accelerated = PIKA adjuvant rabies vaccine accelerated regimen; GMT = Geometric mean titer; Change = visit value – baseline value; Time to targeted RVNA = duration (days) from the date of randomization to the visit at which RVNA 0.5 IU/ml was reached for the first time.
such a strategy might be of potential clinical benefit and has important clinical implications. We also surmise that in developing countries with poor access to healthcare facilities, a three-day accelerated regimen can make a substantial impact on patient compliance and cost reduction. Whilst the doses might need to be doubled on Days 1 and 3, this would be offset by cheaper production costs as less antigen is needed per vial compared to the current commercially available vaccines.

The findings of this study need to be interpreted in light of its limitations. Assumptions for estimating the required sample size and evaluation of endpoints in this study were based on the design and results of the previous Phase I study, which involved small numbers of subjects. In this study, the observed between-group differences were smaller than in the Phase I study; it is possible that these assumptions may need to be re-examined when designing future trials. Although non-inferiority of the PIKA rabies vaccine to the commercial vaccine was demonstrated, it was not possible to assess superiority as all subjects attained the target RVNA level by Day 14. In addition, the PIKA and commercial vaccine were administered using differing regimens and we acknowledge that we may have observed a different result if both vaccines were administered using a similar regimen. Nonetheless, head-to-head comparison of the Essen regimen using the PIKA vaccine and the control vaccine in our earlier phase 1 study showed a higher proportion of the PIKA vaccine subjects achieving a RVNA titer of $\geq 0.5$ IU/ml and having a higher median RVNA titer at day 7 [12].

Stratified randomization based on age and gender was performed to assign subjects to the PIKA-accelerated or control-classic groups; in future studies, it may be necessary to take into account additional baseline factors that could influence the immune response. In this study, we performed the FAVN assay to measure RVNA titers, rather than the rapid fluorescent focus inhibition test (RFFIT) which is the recommended method for testing of human sera by the WHO [17]. Although there is direct correlation between FAVN and RFFIT, the two tests are not completely concordant [18]. Hence for future Phase III studies, we will utilize RFFIT for measurement of RVNA titers. Finally, larger sample sizes would be needed to detect differences between treatments.

Overall, the results of this study have shown that the PIKA rabies vaccine under an accelerated regimen elicited a robust immunogenic response comparable to that of the control vaccine, with production of RVNA as early as seven days after vaccination. No new or clinically important safety concerns were noted in this study and the safety and tolerability profile of the PIKA rabbit vaccine was comparable to that of the control vaccine. Moving forward, a larger Phase III study is urgently needed in order to robustly evaluate the immunogenicity of the accelerated PIKA rabies vaccine regimen, as early production of protective RVNA is critical for effective post-exposure protection against rabies infection.

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Conflicts of interest
Zhongkai Shi is the executive director of clinical development at Yisheng Biopharma (US). Juliahabee A. Kassim and Jing Zhao are both project managers at Yisheng Biopharma (Singapore) Pte. Ltd. The study was conducted by parties that have no commercial interest in Yisheng Biopharma (Singapore) Pte. Ltd., nor any commercial interest in investigational PIKA rabies vaccine, nor the outcome of the study other than specified in the terms and conditions in the respective service agreements for completion of the clinical trial work.

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Appendix A. Supplementary material
Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2017.10.097.

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