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An accelerated rabies vaccine schedule based on toll-like receptor 3 (TLR3) agonist PIKA adjuvant augments rabies virus specific antibody and T cell response in healthy adult volunteers

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ABSTRACT

Background: Rabies is a fatal disease where post-exposure prophylaxis (PEP) is crucial in preventing infection. However, deaths even after appropriate PEP, have been reported. The PIKA Rabies vaccine adjuvant is a TLR3 agonist that activates B and T cells leading to a robust immune response.
Methods: We conducted a phase I, open label, randomized study in healthy adults to assess the safety and immunogenicity of the PIKA Rabies vaccine and an accelerated vaccine regimen. Thirty-seven subjects were randomized into 3 groups: control vaccine classic regimen, PIKA vaccine classic regimen and PIKA vaccine accelerated regimen. Subjects were followed up for safety, rabies virus neutralizing antibodies (RVNA) and T cell responses.
Results: Both the control and PIKA Rabies vaccine were well tolerated. All adverse events (AEs) were mild and self-limiting. Seventy-five percent of subjects in the PIKA accelerated regimen achieved a RVNA titer $\geqslant$0.5 IU/mL on day 7, compared to 53.9% in the PIKA classic regimen ($p = 0.411$) and 16.7% in control vaccine classic regimen ($p = 0.012$). The PIKA rabies vaccine elicited multi-specific rabies CD4 mediated T cell response already detectable *ex vivo* at day 7 after vaccination and that was maintained at day 42.
Conclusion: The investigational PIKA rabies vaccine was well tolerated and more immunogenic than the commercially available vaccine in healthy adults.
Clinical trial registry: The study was registered with clinicaltrials.gov NCT02657161.

1. Background

Rabies is a zoonosis, transmitted from animal to human via a contaminated wound with saliva-borne rabies virus. It is a lethal infectious disease [1–5], with an estimated 60,000 deaths annually - 95% of which occurs in Asia and Africa [6]. Disease is preventable by immediate local wound treatment and PEP [3]; occasionally co-administration of rabies immunoglobulins (RIG) is needed [7].

PEP induces antibodies against rabies virus, hence a full course should be administered as soon as possible. The two common PEP regimens are the Essen (four injections administered on days 0, 3, 7, 14) [8] and the Zagreb schedule (two injections administered on day zero and one injection on days 7 and 21 each) [9]. In the Zagreb schedule, antibody titers of 0.5 IU/mL were achieved in all patients by day 14 [10].

Abbreviations: ACIP, Advisory Committee on Immunization Practices; AEs, adverse events; CSIRO, Commonwealth Scientific and Industrial Research Organization; CTCAE, Common Terminology Criteria for Adverse Events; ELISPOT, enzyme-linked immunosorbent spot; FAVN, Fluorescent antibody virus neutralization; GlyRab, rabies glycoprotein; IPRV, Inactivated and Purified Rabies Virus; PBMCs, Peripheral Blood Mononuclear cells; MAbs, monoclonal antibodies; MedDRA, Medical Dictionary for Regulatory Activities; mITT, modified intent-to-treat; PEP, post-exposure prophylaxis; PIKA, Polyinosinic-Polycytidylic Acid Based Adjuvant; PP, per-protocol; RIG, rabies immunoglobulins; RVNA, rabies virus neutralizing antibodies; SAEs, Serious Adverse Events; SFU, spot-forming units; TLR3, Toll-like receptor 3; WHO, World Health Organization.

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However, in spite of PEP, death after vaccination occurs. This is likely due to extremely short incubation periods from severe bites to highly innervated areas like face, neck or hands before a protective immune response can be achieved by vaccination to protect against disease onset and death.[11,12]. Thus, an accelerated vaccine regimen after exposure may potentially improve vaccination efficacy. The PIKA adjuvant is a synthetic analogue of a dsRNA and a refined form of Polyinosinic-Polycytidylic Acid stabilized with kanamycin and calcium [13–15]. As a TLR3 agonist, it activates potent antigen presenting cells like dendritic cells, leading to a more robust adaptive immunity [14,15].

Mice challenged with a lethal rabies dose were fully protected when treated with PIKA vaccine and achieved higher IgM and IgG titers after immunization compared to aluminum adjuvant [16]. Recent studies of infected mice also showed that PIKA rabies vaccine administered using an accelerated regimen enhanced survival in mice from 67.7% to 80% compared to the standard regimen [17].

Thus, in this study we sought to address the compelling medical need for a more potent, rapid and robust post exposure vaccine with a shorter vaccine regimen and higher immunogenicity that would be beneficial. We evaluated the investigational PIKA rabies vaccine for its B and T cell immunogenicity, described its safety profile compared with a commercial rabies vaccine and further tested an accelerated regimen to evaluate its ability to induce earlier and higher titer of RVNA, and T cell mediated immunity in the early stages of rabies infection.

2. Material and methods study design

This was a Phase I, single-centre, open label, randomized study which involved healthy adult subjects aged 21–65 years in Singapore, with no prior history of rabies vaccination and undetectable RVNA at baseline. The study was designed to assess the safety, tolerability and immunogenicity of the PIKA rabies vaccine as well as the immunogenicity of an accelerated vaccine regimen. Study approval was obtained from Singapore Health Sciences Authority (HSA CTC1400532) and Centralized Institutional Review Board (CIRB Ref: 2014/747/F). The study was performed in agreement with the International Conference on Harmonization guidelines on Good Clinical Practices, laws and regulatory requirements in Singapore. Informed consent was obtained from each subject prior to screening. Subjects were first enrolled on 02 February 2015 with the last subject visit on the 23 July 2015.

Forty-four subjects were screened with 37 subjects randomized into three groups at a ratio of 1:1:1; using randomly generated numbers in pre-sealed envelopes. In order to have at least 10 healthy volunteers reaching the primary end point in each of the three groups, it was planned to recruit 12 per group for a total of 36 subjects. This was based on an estimate of 20% dropout rate from the recruited cohort. The RVNA titer levels were batched tested in Commonwealth Scientific and Industrial Research Organization (CSIRO)-Australian Animal Health Laboratory at the end of the last subject visit. Subject disposition in the study is illustrated in Fig. 1a.

Group A received the control vaccine, a commercially available rabies vaccine (Novartis RABIPUR®) (Batch No.: 548011H) containing 5.9 IU/ vial of inactivated Rabies virus), while Group B received the investigational PIKA rabies vaccine (containing 2.0 IU of inactivated Rabies virus). Both groups were vaccinated using the classic 4-dose regimen (1–1–1–1), whilst Group C received the investigational PIKA rabies vaccine with an accelerated regimen (2–2–1). (Fig. 1b)

The primary endpoints were safety and immunogenicity response, induced by the PIKA rabies vaccine. All AEs were captured in the three groups. Safety data was recorded throughout the study, up to day 42. Immunogenicity was measured using the RVNA titer level from serum at Day 0, 7, 14 and 42 after the first vaccination, with a level of at least 0.5 IU/ml according to World Health Organization (WHO) requirement, as evidence for protection.

The secondary endpoints were detectable specific T cell mediated immune response on day 0, 7, 14 and 42 and the RVNA titer on day 0, 7, and 14 and 42 in the accelerated regimen compared to the classic regimen with control vaccine. Rabies-specific T cell response at Day 0, 7, 14 and 42 were tested directly *ex vivo* using ELISPOT assay.

2.1. Subjects and study procedures

Participants 21 to 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 were collected on structured case report forms and entered into a secure, web-based database maintained by Clinactis Pte. Ltd., a commercial clinical research organization. Investigators had no access to the database until completion of the trial.

2.2. Vaccine production

The investigational PIKA rabies vaccine was manufactured by Liaoning Yisheng Bio-Pharmaceutical Co., Ltd in Daxiner Village, Cailuo Town, Xinchengzi District, Shenyang in China. These facilities meet the GMP standards for quality assurance and control.

The CTN-1 rabies virus strain was obtained by isolating a strain of wild virus from the brain tissue of a human rabies death case in Shandong, China in 1956 (named CTN-1S), It was passaged in Vero cell lines, which are the most commonly used cell culture for rabies vaccine today [18,19].

The investigational PIKA rabies vaccine is a combination of the Inactivated and Purified Rabies Virus (IPRV) (CTN-1 strain virus antigen that is being produced in Vero cell line) and the PIKA adjuvant combined by mixing in a phosphate buffer solution. The ratio of antigen and adjuvant mix has been optimized to reduce the dose without compromising the potency in pre-clinical animal tests. 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 of 2.0 IU of inactivated purified rabies antigen; 1.0 mg of PIKA adjuvant; 3ug of human albumin; 30ug of maltose and 50ug of dextran.

2.3. Safety assessments

Monitoring for adverse events included a medical assessment within 2 h prior and post vaccination. Systemic and local injection site AEs were documented by study personnel up to 2 h after each injection. All subjects were provided with a patient diary to record all solicited local and systemic AEs and any other unsolicited AEs up to day 42. Pre-specified haematology and biochemistry blood tests and urine analyses were taken at all study visits. (Supplementary Tables 1 and 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.

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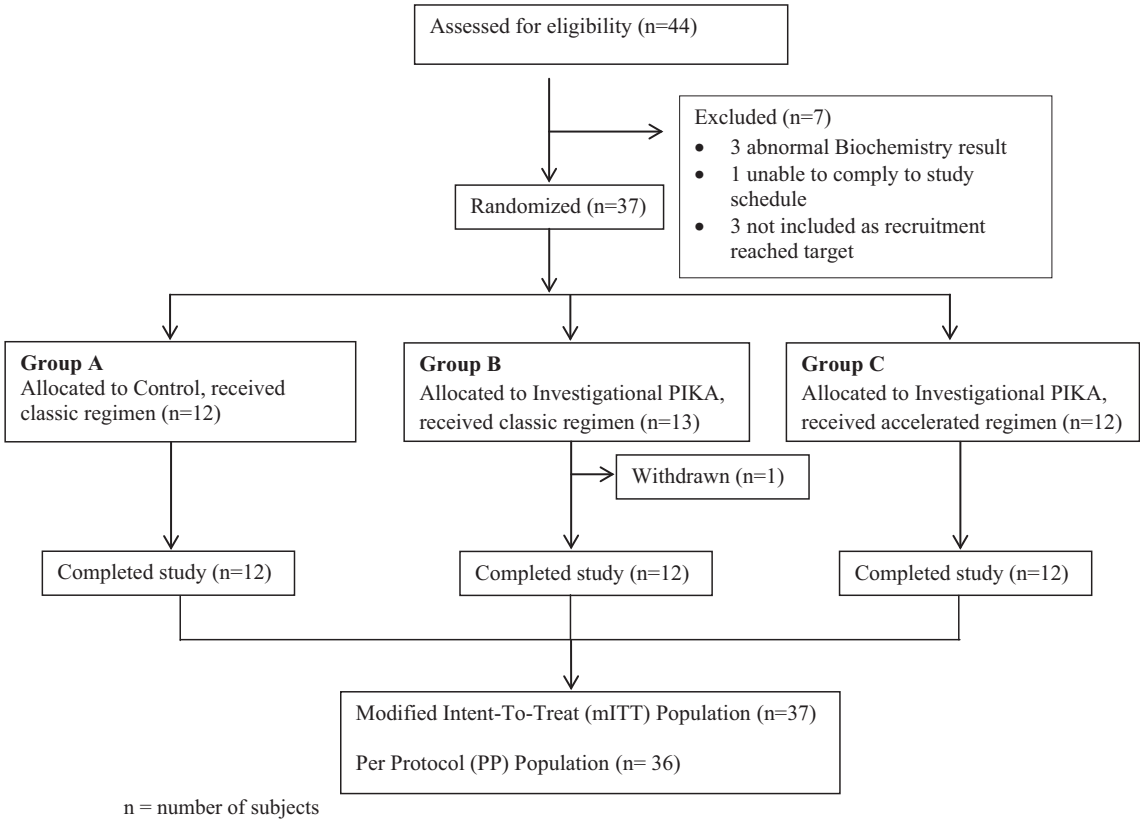

Fig. 1a. Schematic diagram of study design, screening enrollment and follow up of participants.

3 Groups

Group A: Control Vaccine

Group B: PIKA Vaccine

Group C: PIKA Vaccine (accelerated vaccine regimen)

Group	Subjects completed study	Vaccine	Regimen (Intramuscular)	Study (Day)			
				0	3	7	14
A	12	Control vaccine	1-1-1-1	×	×	×	×
B	12	PIKA rabies vaccine	1-1-1-1	×	×	×	×
C	12	PIKA rabies vaccine	2-2-1	×	×	×	

Note: Vaccination - ×

Fig. 1b. Vaccine Regimens in all groups.

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

The endpoint for assessing the humoral immune response was the serum RVNA titer at Day 14 and 42 after the first injection, with a titer of 0.5 IU/mL meeting protection, in accordance with WHO requirements. RVNA titer was determined by the Fluorescent antibody virus neutralization (FAVN) test performed at CSIRO-Australian Animal Health Laboratory [20]. Current ACIP recommendations consider a complete neutralization at a serum dilution of 1:5 in FAVN testing an adequate response to rabies vaccination.

2.5. Synthetic peptides

A panel of 100 15-mer peptides overlapping by 10 amino acid (aa) residues were used to test Rabies Glycoprotein (GlyRab) - specific T cell responses. The peptides covered the entire sequence of the GlyRab (GenBank accession number AY009100.1) and were purchased from China Peptides. The purity of the peptides was greater than 95% and their composition was confirmed by mass spectrometry analysis. They were pooled in a 13 by 8 matrix, containing thirteen or eight peptides/pool using a concept similar to what was previously described for HIV [21].

2.6. Isolation of PBMC and in vitro expansion of rabies specific T cells

Peripheral Blood Mononuclear cells (PBMCs) from subjects were isolated by Ficoll-Hypaque density gradient centrifugation and resuspended in AIM-V medium with 2% pooled human AB serum. Cells were used directly *ex vivo* and/or after a 10-day antigen specific *in vitro* stimulation in the Enzyme-Linked Immunosorbent Spot (ELISPOT) IFN-γ assay using 100 overlapping peptides covering the GlyRab sequence to analyze for rabies-specific T cell responses Rabies mortality still remains high despite the use of current rabies vaccines that induce RVNA upon timely PEP administration. Mortality is likely due to delayed generation of a specific humoral and cellular immunity that are critical for containing the spread of rabies virus infection especially in highly innervated areas of the body. The development to a more immunogenic vaccine against rabies virus is needed [22].

2.7. Intracellular cytokine staining (ICS)

In vitro-expanded PBMCs were incubated in medium alone (control) or GlyRab peptides (5 μg/ml) for 16 h in the presence of brefeldin A (2 mg/ml). After washing, the cells were stained with anti-CD8 phycoerythrin (PE)-Cy7, anti-CD4 Brillant Violet 650 (BV650) monoclonal antibodies (MAbs) for 30 min at 4 °C and then fixed and permeabilized using Cytofix/Cytoperm Fixation/Permeabilization solution. Cells were then stained with anti-IFN-γ Allophycocyanin (APC), anti-TNF-alpha Alexa Fluor 488 (AF488), IL-2 PE for 30 min on ice, washed, and analyzed by flow cytometry.

2.8. Statistical methods

The study was not intended to reach prior statistical requirements, and the sample size was customary for Phase 1 studies primarily evaluating safety parameters. Safety data was summarized using descriptive statistics, with laboratory measurements and changes detected between each dose group to control group using an analysis of variance (ANOVA) model. For each dose group and each measurement time point post-dose, mean change compared to control group, with 90% confidence interval, was calculated.

3. Results

3.1. Demographic characteristics and enrollment

There were no significant differences in the baseline demographics of the subjects enrolled as shown in Table 1. A predominance of male subjects was observed in each group (66.7% in Group A, 76.9% in Group B, 83.3% in Group C). The mean age of the enrolled subjects in Groups A, B and C were 31 years, 37 years and 39 years respectively. Most of the subjects were of Chinese origin (91.7% in Group A, 76.9% in Group B, 83.3% in Group C), consistent with the ethnic distribution in Singapore.

3.2. Safety

Overall, both the control and investigational vaccines in the classic or accelerated regimen were well tolerated. Twenty subjects experienced a total of 47 AEs over the course of the study (Table 2). All AEs were mild in severity. No severe AEs or deaths were reported. AEs in the pooled modified intent-to-treat (mITT) population were similar to those in the per-protocol (PP) population. There were no significant differences in AEs related to study drug in each group (A vs B ($p = 0.6951$), A vs C ($p = 1.0000$), B vs C ($p = 1.0000$)) (Supplementary Table 3).

Twenty-six systemic reactions were observed in 16/37 (43.2%) subjects; among these, 04/12 (33.3%), 08/13 (61.5%), 04/12 (33.3%) were from Group A, B and C respectively (Supplementary Table 4). Asymptomatic pyuria was the most frequent systemic reaction followed by presence of glucosuria, headache, diarrhoea, lethargy and proteinuria. Pyuria, occurred in 02/13 (15.4%) and 01/12 (8.3%) subjects from Group B and C respectively. They were graded as mild and unrelated to study treatment (Group B) or possibly related (Group C). The AEs resolved after the last treatment visit.

Twenty-one solicited injection site reactions were reported in 10/37 (27.0%) subjects. The total number of subjects with at least one local AE for Groups A, B and C are 01/12 (8.3%), 06/13 (46.2%) and 03/12 (25.0%) respectively (Supplementary Table 5). The most frequently reported local AE following administration of vaccine was injection site pain in 06/13 (46.2%) and 03/12 (25%) subjects in Groups B and C respectively. The AEs were graded as mild and definitely related to study treatment. None of the subjects with solicited injection site reactions required treatment.

None of the AEs led to discontinuation of the study except for one subject in Group B. The subject had received 3 doses of PIKA rabies vaccine and was withdrawn by the study investigator at visit 5 due to pruritus. There was no associated rash or other systemic involvement. The pruritus. resolved after 16 days with the AE classified as probably related to the study treatment.

3.3. Ability of different vaccine preparations to elicit RVNA production

Serum RVNA level of at least 0.5 IU/ml obtained at day 0, 7, 14 and 42 after the first injection was compared across the 3 groups using the PP population. At day 7, the highest number of subjects achieving RVNA titer of 0.5 IU/mL were from Group C (09/12, 75.0%), compared with Group A (02/12, 16.7%) and Group B (07/13, 53.9%). (Table 3a) There was a significant difference in number of subjects achieving a titer of $\geqslant$ 0.5 IU/mL between Groups A and C ($p = 0.0123$) (Table 3a). All the subjects in the three groups achieved a RVNA titer of $\geqslant$0.5 IU/mL by day 14 (Supplementary Fig. 1 and Table 3a).

Group C had a higher median RVNA titer at day 42 compared to Group A and B (Table 3b), suggesting that the accelerated PIKA

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Table 1
Baseline demographics by study group.

	Group A (N = 12)	Group B (N = 13)	Group C (N = 12)
Age at enrolment (years)			
Mean ± SD	31 ± 7	37 ± 8	39 ± 12
Minimum; maximum	22, 42	25, 47	22, 63
Gender: n (%)			
Male	8 (66.7%)	10 (76.9%)	10 (83.3%)
Female	4 (33.3%)	3 (23.1%)	2 (16.7%)
Ethnic origin: n (%)			
Chinese	11 (91.7%)	10 (76.9%)	10 (83.3%)
Indian	0 (0.0%)	1 (7.7%)	1 (8.3%)
Malay	1 (8.3%)	2 (15.4%)	1 (8.3%)
Others	0 (0.00%)	0 (0.0%)	0 (0.0%)
Mean ± SD height (m)	1.67 ± 0.09	1.68 ± 0.09	1.70 ± 0.07
Mean ± SD weight (kg)	64.5 ± 14.67	65.6 ± 13.30	71.1 ± 15.76

Table 2
Overall Summary of Adverse Events by study group.

	Group A (N = 12) n (%)	Group B (N = 13) n (%)	Group C (N = 12) n (%)
Total number of AE	5	33	9
Total number of subject with at least one AE	5 (41.7%)	9 (69.2%)	6 (50.0%)
Total number of SAE	0	0	0
Total number of subjects with SAE	0 (0.0%)	0 (0.0%)	0 (0.0%)
Deaths	0 (0.0%)	0 (0.0%)	0 (0.0%)
Subjects discontinued due to AE/SAE	0 (0.0%)	1 (7.7%)	0 (0.0%)

rabies vaccine regimen was able to sustain high RVNA titers even at day 42.

3.4. Induction of rabies-specific T cell immune response by different vaccination schedule

The presence of rabies-specific T cells were assessed on day 0, 7, 14 and 42 in all subjects ex-vivo using a library of 100 synthetic overlapping peptides covering the whole GlyRab used to stimulate PBMCs of the healthy subjects in an ELISPOT IFN-γ assay. The rabies-specific response was considered positive when the number of spot-forming units (SFU) was more than 5 and at least two times above the mean of the unstimulated control wells. An example is shown in Fig. 2b.

Overall, the PIKA accelerated vaccination schedule was the most efficient. GlyRab specific T cells were detectable at day 7 only in Groups A 03/12 (25%) and C 05/12 (42%). At day 14, positive GlyRab specific T cell responses were detected in 09/12 (75%) from Group C, 02/13 (15%) in Group B and 06/12 (50%) in Group A. Even though the differences were not statistically significant, subjects in the PIKA accelerated group elicited a more robust (Fig. 2b) and multi-specific (Fig. 2c) GlyRab T cell response than the other 2 groups. More importantly, the presence of GlyRab-specific T cells in subjects at day 42 show that only PIKA vaccinations (Groups B and C) can induce rabies-specific T cells that are still detectable ex-vivo at least one month after their last vaccination.

GlyRab-specific T cell responses were compared after *in vitro* expansion in five subjects from each groups. This confirmed that the direct *ex vivo* responses previously detected and the GlyRab-specific T cell frequency is similar in both direct *ex vivo* and *in vitro* expanded cells (Fig. 3a). To confirm that the ex-vivo ELISPOT assay were able to detect rabies-specific T cells and to characterize whether such responses were supported by multifunctional helper (CD4) or cytotoxic (CD8) T cells, we produced rabies-specific T cells using all of the overlapping glycoprotein peptides stimulated *in vitro*. The antigen specific T cells were subjected to ELISPOT IFN-γ assay then analyzed using intracellular cytokine staining. Using the ELISPOT matrix, we tested whether the GlyRab-specific T cells upon single peptide stimulation, were of CD4 or CD8 origin and their ability to produce IFN-γ, TNF-α and IL-2. We confirmed the presence of T cells specific for 25 single GlyRab peptides (peptides list in supplementary Table 6). All GlyRab-specific T cells induced by the different vaccinations were CD4+ T cells and showed similar ability to produce single or multiple cytokines upon single peptide stimulation (Fig. 3b).

Table 3b
Summary of RVNA titer level by study group in the per protocol population .

Visit (Day)	Group A (N = 12)	Group B (N = 12)	Group C (N = 12)
Visit 2 (Baseline)			
Median	0.08	0.08	0.08
Range	0.07–0.20	0.08–0.35	0.08–0.29
Visit 4 (Day 7)			
Median	0.22	0.35	0.60
Range	0.08–0.79	0.08–9.36	0.07–1.37
Visit 5 (14 ± 2 Days)			
Median	22.92	12.91	30.17
Range	3.12–48.64	5.92–48.64	5.40–48.64
Visit 6 (42 ± 5 Days)			
Median	3.81	9.03	10.26
Range	0.50–36.96	1.97–36.96	1.50–121.50

4. Discussion

Rabies mortality still remains high despite the use of current vaccines that induce RVNA upon timely PEP administration. Mortality is likely due to delayed generation of a specific humoral

Table 3a
Proportion of subjects reaching ⩾0.5 IU/ml RVNA by study day.

Study day	Responder	Group A (N = 12)	Group B (N = 13)	Group C (N = 12)	p-value A vs. B	p-value A vs. C	p-value B vs. C
Day 7	Yes	2 (16.7%)	7 (53.9%)	9 (75.0%)	0.0968	0.0123	0.4110
	No	10 (83.3%)	6 (46.2%)	3 (25.0%)			
Day 14 ± 2	Yes	12 (100.0%)	13 (100.0%)	12 (100.0%)			
	No	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Day 42 ± 5	Yes	12 (100.0%)	13 (100.0%)	12 (100.0%)			
	No	0 (0.0%)	0 (0.0%)	0 (0.0%)			

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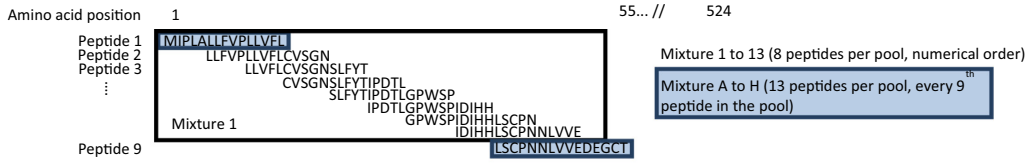
(A)



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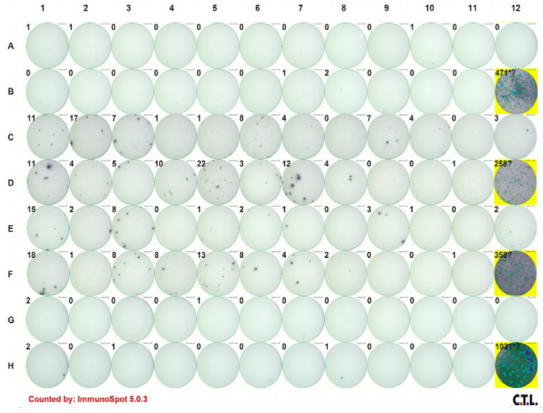

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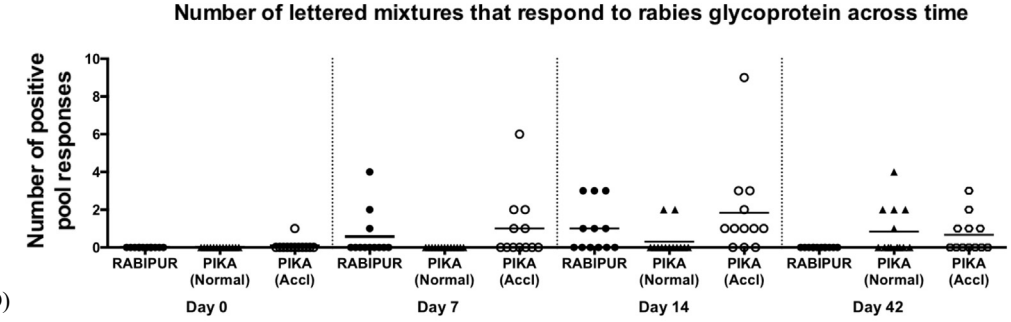

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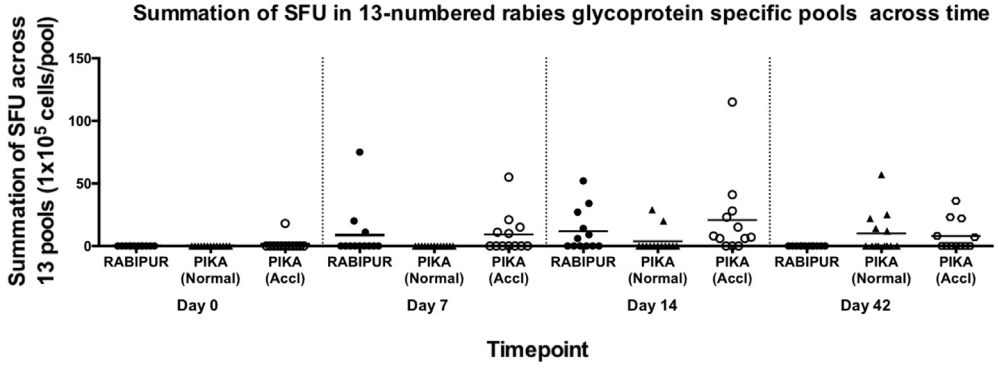

Fig. 2. (a) Schematic of ELISPOT matrix along the whole of the rabies glycoprotein sequence. 15-mer peptides overlapping by 10 amino acid (aa) residues were made along the whole sequence from position 1 to 524. The matrix is arranged into a 13-by-8. Mixture 1–13 were arranged in numeric order, containing 8 peptides in each mixture. Mixture A to H contains 13 peptides, and arranged with every 9th peptide from the library of peptides. (b) A representative of ELISPOT plate that was developed direct *ex-vivo* using $1x10^5$ PBMCs per well, incubated with the rabies glycoprotein ELISPOT matrix pools. The schematic below shows the arrangement of the ELISPOT matrix pools, containing both the numbered and alphabetical pools with duplicates of negative control and a positive control. The numbers on the plate refers to the number of spot forming units (SFU) that are counted using the ImmunoSpot software. (c) Quantification of the number of subjects responding to one or more of the IFN-γ ELISPOT matrix pools in each of the vaccination arms across the vaccine schedule. Each bar represents the number of subjects with positive responses to the mixture covering the numbered region of the rabies glycoprotein. The threshold of positivity is positive when the number of spot-forming units (SFU) was more than 5 and at least two times above the mean of the unstimulated control wells. Each symbol represents the number of positive responses from each individual subject. (d) Quantification of SFU across the 13 numbered IFN-γ ELISPOT matrix pools in the vaccination arms across the vaccine schedule. Each symbol represents the summation of SFU across the 13 numbered pools of each individual subject. The same positivity criteria is applied here.

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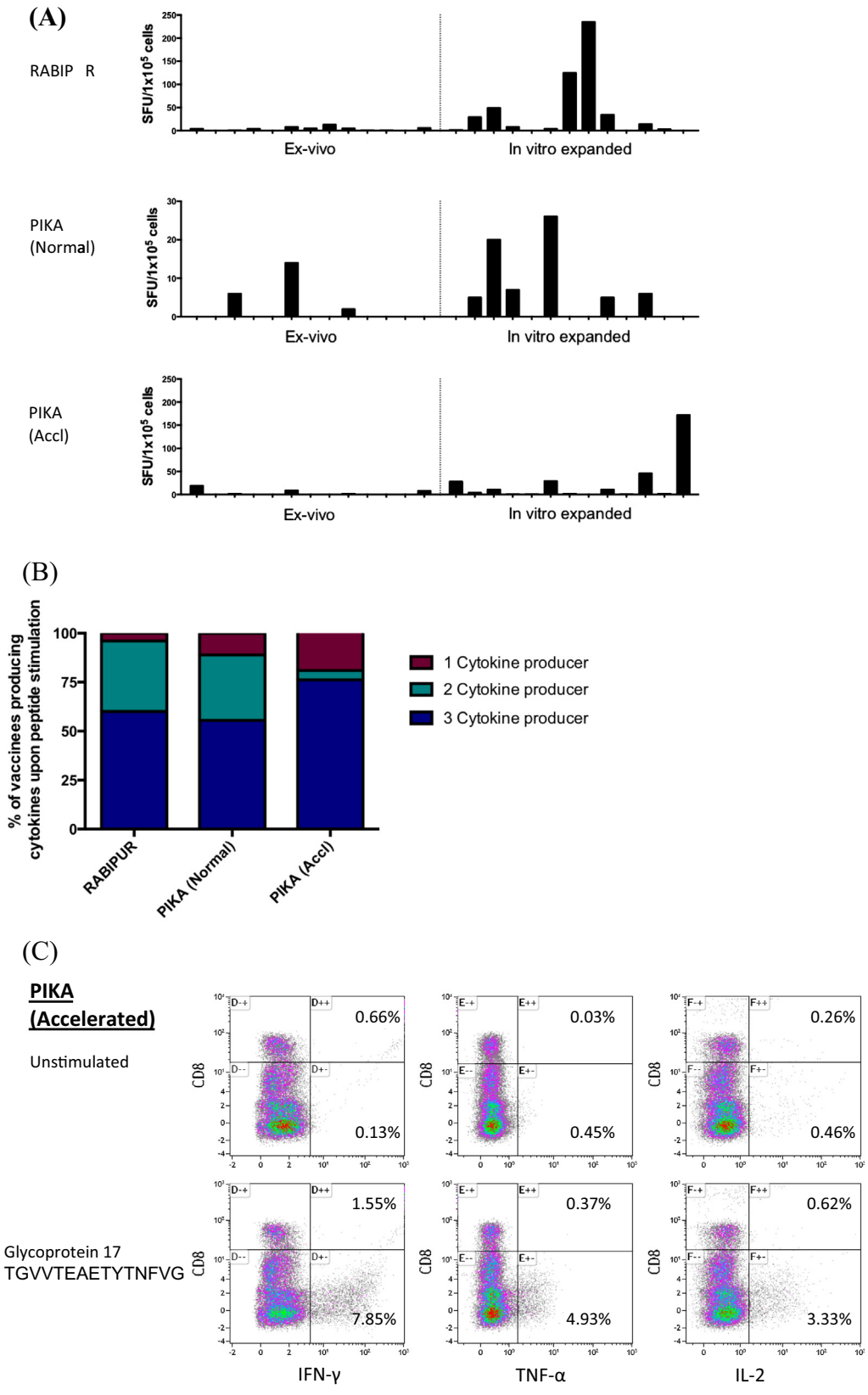

Fig. 3. (a) Representative subjects from each of the vaccination groups showing SFU/1 × 10^5 PBMCs per well across the 13-numbered matrix pools direct *ex vivo* and *in vitro* expanded from the same subject. Each bar represents one of the 13 numbered pools in the IFN-γ ELISPOT assay. (b) Percentage of GlyRab-specific T cells upon single peptide stimulation in an overnight ICS assay producing IFN-γ, TNF-α or IL-2. Cytokine co-expression subsets are expressed as a percentage of total cytokine-producing GlyRab-specific T cells. Single producers, IFN-γ+, TNF-α+ or IL-2+; double producers, IFN-γ + TNF-α+, IFN-γ + IL-2+ or IL-2 + TNF-α+; triple producers, IFN-γ + IL-2 + TNF-α+. Figure C shows a representative dot plot of intracellular cytokine staining with GlyRab-specific T cells that have been *in vitro* expanded with the entire rabies glycoprotein and tested with the individual peptides for IFN-γ, TNF-α and IL-2 production. (c) Representative dot plot from a GlyRab-specific T cells upon single peptide stimulation in an overnight ICS assay. Single peptide of 5 μg/ml concentration was added and stimulated for 16 h before ICS was carried out. GlyRab-specific T cells were stained for CD8, IFN-γ, TNF-α and IL-2.

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and cellular immunity that are critical for containing the spread of infection especially in highly innervated areas of the body. The development of a more immunogenic vaccine against rabies virus is needed.

This study was designed to compare the safety and immunogenicity of a new Rabies PIKA vaccine with the RABIPUR vaccine that conforms to WHO standards, confirming the preclinical and clinical studies of previously reported safety and tolerance of the PIKA vaccine [16,23]. PIKA vaccine and RABIPUR control groups have comparable AEs with the commonest AE related to immunization; pain at the injection site. Most of these reactions resolved within 48 h post vaccination with no SAEs reported. Overall, the safety profile of PIKA rabies vaccine is non-inferior to the control RABIPUR vaccine.

The analysis of immunogenicity shows that PIKA vaccine induced serological and cellular immune responses towards the rabies virus is more efficient than those induced by RABIPUR vaccination and confirms the results obtained in animal models. All vaccinations schedules trigger an antibody response that was present in all subjects at day 14. However, PIKA accelerated group has 75% of subjects with 0.5 IU of RVNA titres as early as day 7 compared to 16.7% in RABIPUR group. Despite all vaccinated individuals reaching the WHO recommended protection of 0.5 IU, the median RVNA titres were significantly higher in the PIKA accelerated group than the other two groups. This trend in B cell responses was also observed in the T cell responses where PIKA accelerated group has a higher GlyRab specific T cell responses starting from day 7. It is important to note that the antigenic quantity of rabies virus in the two vaccine preparations were 2.0 IU per dose of PIKA vaccine and 5.9 IU per dose in the batch of RABIPUR vaccine. Thus even in the accelerated PIKA regimen, where subjects received two simultaneous vaccine doses, the quantity of rabies antigen introduced was lower than the quantity used in RABIPUR vaccinated subjects. Furthermore, although mandated by WHO that the antigen content requires a minimum of 2.5 IU, the PIKA vaccine containing only 2.0 IU per dose, induced a robust serological and cellular immune responses towards the rabies virus and maintained a detectable cellular response at even day 42. Moreover, PIKA accelerated regimen elicited a more robust and multi-specific GlyRab T cell response compared to the other two groups (Figs. 2b and 3c).

Different variables in addition to the antigenic quantity can affect the immunogenicity of the two different vaccine preparations namely, (a) the source of rabies antigen production and (b) the adjuvant used. The scope of this work was not to disentangle the exact interplay that contributes to the efficacy of the PIKA vaccine, but only to directly test in healthy subjects their overall ability to induce a rabies-specific immunity. Nevertheless, the source of rabies antigen of RABIPUR and the PIKA vaccine is different, namely from primary chicken fibroblasts cell culture and Vero cell line respectively. Both sources are well documented and have been licensed for use in different countries (RABIPUR licensed in globally while Vero cell based vaccines have been licensed in Europe and China). Thus, the possibility that rabies antigen produced in different sources have different immunogenicity cannot be discounted. It is however more likely that the increased immunogenicity of the PIKA-rabies vaccine was due to the PIKA rabies adjuvant that has been reported in pre-clinical studies of mice to enhance protection by inducing humoral and cellular immunity as compared to an aluminium adjuvanted vaccine [16]. PIKA has previously been described to promote maturation of dendritic cells and can induce cytokines such as IL-12p40 and IL-6 [15]. Alum that is formulated with the RABIPUR vaccine is an adjuvant that promotes mainly humoral responses [24], coupled with the fact that RVNA readout is the sole parameter to measure immunogenicity of a rabies vaccine. We postulate that the combined synergy from the PIKA adjuvanted vaccine and the vaccination schedule are likely the main drivers for the superior immunogenicity observed in the PIKA vaccinated subjects.

In conclusion, our data indicate that vaccination with PIKA on an accelerated schedule can elicit better immunological responses towards the rabies virus in both the serological and cellular arms. This could translate to a rapid ability to inhibit viral replication at the site of infection to prevent and control the spread of infection, a critical parameter in decreasing PEP failures and fatalities.

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Conflict of interests

LT Li is the co-founder and executive director of 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 services 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 http://dx.doi.org/10.1016/j.vaccine.2016.12.031.

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