



Efficacy of ormutivimab, a novel recombinant human anti-rabies monoclonal antibody, in post-exposure prophylaxis animal models

Li-li zhai^a, Hui Wang^a, Wei Zhao^a, Shou-feng Zhang^b, Fa-ming Miao^b, Yang Cao^a, Chen Chen^a, Yu-Feng Li^a, Jie Gao^a, Ruo-yun Lv^a, Shi-xiong Zhang^a, Jia-bin Cao^a, Xu-fan Zhang^a, Ming-ming Yang^a, Bin Zhang^a, Jing Zhao^a, Jing-shuang Wei^{a,*}, Jian Gao^{a,**} gaojian3993@aliyun.com

^a NCPC New Drug Research and Development Co., Ltd., State Key Laboratory of Antibody Research & Development, Shijiazhuang, 052165, Hebei Province, China

^b Laboratory of Epidemiology and Key Laboratory of Jilin Provincial Zoonosis Control and Prevention, Military Veterinary Research Institute, Academy of Military Medical Sciences, Changchun, China

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ABSTRACT

Human rabies is a serious public health problem that can't be ignored. Rabies immune globulin (RIG) is an indispensable component of rabies post-exposure prophylaxis (PEP). However, current PEP relies on RIG purified from pooled human or equine plasma, which are either in chronic shortage or associated with safety concerns. Monoclonal antibodies have become widely accepted as safer and more cost-effective alternatives to RIG products in recent years. Here, we assessed the neutralization breadth of human monoclonal antibody ormutivimab and its protective efficacy in PEP models. Ormutivimab was able to neutralize a broad panel of Chinese prevalent street RABVs with neutralizing potency from 198–1487.6 IU/mL. Furthermore, ormutivimab offered comparable protection to that with HRIG both at standard doses (20 IU/kg) and higher doses (100 IU/kg and 200 IU/kg). The interference of ormutivimab on vaccine potency was also analyzed and found slightly reduced neutralizing antibody titers similar to HRIG. The broad-spectrum neutralization activities, highly protective potency, and rapid onset of action make ormutivimab an effective candidate for human rabies PEP.

1. Introduction

Rabies continues to pose a serious threat to public health, especially in developing countries. Although progress has been made in rabies prevention, the disease control is still a major challenge. Approximately 15–29.2 million people globally receive post-exposure prophylaxis (PEP) each year, and it has been reported that about 60% of PEP recipients are category III exposed [1]. Human rabies death in various areas of the world in 2010 is to be from 26,400 to 61,000 [2,3]. Among those, India was reported to have the highest incidence with around 20,565 human death in 2003 [1,4] and the second is in China with about 202 human death in 2020 [5]. Due to the poor surveillance and under-reporting in many developing countries and other factors, the scale of the disease burden are likely to underestimation [6].

Rabies prevention in humans is achieved by PEP if administered promptly and appropriately. Traditional rabies immune globulins

(RIGs), including equine anti-rabies immune globulin and human anti-rabies immune globulin (HRIG), are currently used for human rabies PEP. Although highly effective, RIGs have some limitations. They were associated with adverse effects, such as serum sickness (1–3% of recipients) and anaphylactic reaction (1/150,000) [1,7,8]. Possibilities of contamination by unknown pathogens is another risk for RIGs. Moreover, because of the expense and lack of availability, only 1–10% of category III exposed patients actually have access to this life-saving product [1,7]. It has been reported that for a patients with 60 kg body weight, the cost for HRIG ranged from \$US 250 to \$US 500, which represents 75 d to 150 d of wages for an Indian laborer [1]. Additionally, HRIG, polyclonal derived from human blood, may include other neutralizing antibodies which may affect efficacy and lead to unknown results. Rabies mAbs have been considered as a viable and promising option to address the limitations of blood derived RIGs [7,9–17] in light of the fact that rabies mAbs would have the advantage in term of

* Corresponding author.

** Corresponding author.

E-mail address: wjs3043@sina.com (J.-s. Wei).

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specificity, quality control, minimal allogeneic reactions and longer *in vivo* half-life [1,17]. To date, there have been two launched mAbs [9,11,18–21], and another two mAbs products are still in advanced clinical trials [22–27]. Rabishield is a single humanized IgG1 type mAb that binds to a conformational epitope of the rabies glycoprotein and Rabi-Mabs combined two murine mAbs (M777-16-3 and 62-71-3) which binds two different site on rabies glycoprotein. Both of these two mAbs products are found to be safe and demonstrated non-inferiority to HRIG in levels of RVNA [28]. Nevertheless, Rabishield has only been launched in India. Humanized anti-rabies antibody SYN023 is now in clinical development in China and The clinical trial of CL184, an antibody produced by Crucell, was recently halted for unknown reasons. Thus, the approval of a safe, effective and available alternative rabies mAbs in China is urgently needed.

To this end, we developed the recombinant human monoclonal antibody ormutivimab previously [29]. The heavy- and light-chain coding regions of the SO57 [15,30] antibody genes were synthesized and introduced into pMH1 plasmid which was granted from AmProtein biotech company. SO57, which has been shown as one of the most potent human antibodies in neutralizing various rabies virus [13], was subsequently included in a cocktail of three human antibodies, SOJA, SOJB and SO57 [15]. However, SO57 was expressed by using Rhabdoviral antibody system, which involved error-prone rhabdoviral RNA polymerase. Therefore it's less suitable for producing consistent and high-quality antibodies. The human mAb ormutivimab was produced by Chinese hamster ovary (CHO) cells, which is the most frequently applied host cell system for industrial therapeutics. Here, we aimed to examine the neutralization breadth and potency of ormutivimab by using a panel of Chinese prevalence RABVs and its protective efficacy against the lethal rabies virus in different PEP models. We also tested the vaccine potency in presence of ormutivimab in this study. Our findings provided an evidence that ormutivimab is a promising candidate for next-generation PEP following human exposure to rabies.

2. Materials and methods

2.1. Fluorescent antibody virus neutralization (FAVN) test

FAVN was conducted as previously described [31]. The antibody ormutivimab was tested in four replicates. From an initial 1:3 dilution of ormutivimab, serial three-fold dilutions with a final volume of 100 μ L were prepared in 96-well plate. Next, 50 μ L of the selected RABV strains at a concentration of 100 TCID₅₀ was added to each well, and the plate was incubated at 37 °C for 60 min. After incubation, 50 μ L of suspension containing 2×10^4 BHK21 cells were added, and the mixture was allowed to incubate for 48 h at 37 °C with 5% CO₂. Plates were fixed in 80% acetone, dried, and stained with FITC-labelled anti-rabies immunoglobulin and observed using a fluorescent microscope at $\times 100$ magnification. For each sample dilution, four wells were scored as either virus present or virus absent. Ormutivimab titer was calculated from the combined result of four wells using the Spearman-Kärber method [32]. The titer of a standard reference serum diluted to contain 0.5 IU/mL was titrated in each test.

2.2. Isolation of challenge virus

CVS24 is a standard challenge strain. Street RABV strain GZ-1, kept at the Military Veterinary Research Institute, was isolated from a dog in Guizhou, China. GX-1, kept at the Military Veterinary Research Institute, is a street RABV isolated from a dog in Guangxi, China. Street RABV strain *Jian*, kept at the Wuhan Institute of Biological Products, was isolated from a human in Ningxia, China. BD06 (GenBank: EU549783.1) is a street RABV isolated from a dog in Hebei, China in 2006. As a representative of a Chinese epidemic isolate in viral clade I, BD06 has since been widely utilized as a challenge virus [17].

2.3. Biologics

Ormutivimab was produced by North China Pharmaceutical Group New Drug R&D Co., Ltd. HRIG for the Kunming (KM) mice study (lot number: 20100412) was obtained from Wuhan Institute Biological Products Co., Ltd., China. HRIG (lot number: 20180101) for the beagle study (lot number: 20180101) was from Guangdong Shuanglin Biotechnology Co., Ltd., China. Rabies vaccine (lot number: 201704102) for the beagle study was obtained from Liaoning Chengda Biotechnology Co., Ltd., China. Rabies vaccine (Verorab) (lot number: u1652) for the Balb/c mice infection study was obtained from Aventis Pasteur S.A.

2.4. *In vivo* KM mice challenge model

Groups of KM mouse with 10–15 g body mass, 5 male and 5 female in each group, were inoculated with RABVs at 100 LD₅₀/50 μ L via the right semitendinosus muscle on day 0. Four hours later, 50 μ L of ormutivimab, HRIG, or saline was administered respectively into the same site as virus inoculation of each mouse. KM mice were maintained and evaluated for up to 21 days after infection. The brain tissues of dead mice were collected for fluorescent antibody test to confirm RABV infection.

2.5. *In vivo* beagle challenge model

Three-to five-month-old male and female beagles (6.0 ± 1.0 kg) with rabies virus-neutralizing antibody (RVNA) < 0.1 IU/mL were selected and randomly assigned to six groups. Beagles in each group were inoculated with street RABV BD06 at 100,000 MICLD₅₀ (1 mL) via the left biceps femoris on day 0. Ormutivimab or HRIG at 20 IU/kg with or without vaccine was administered 6 h after challenge. One human dose of rabies vaccine was immediately administered to RIG + vaccine groups via the right biceps femoris. Four more single doses of rabies vaccine were administered on day 3, 7, 14, and 28 to the RIG + vaccine group. Test animals were examined twice daily for clinical signs of rabies. Brain tissues of dead beagles were collected for immunofluorescence staining of viral antigens to confirm RABV infection. At the end of experiment on day 90, survivors with RAVN ≤ 1.0 IU/mL were euthanized. Euthanasia was achieved through intramuscular injection of anesthetics for deep sedation. Blood was collected at assigned days, and RVNA titers in serum samples were determined by the standard FAVN.

2.6. *In vivo* balb/c mice challenge model

Groups of female Balb/c mice with 16–18 g body mass were inoculated with street RABV *Jian* in the semitendinosus muscle on day 0. Ormutivimab or HRIG was administered into the same site as the virus inoculation site after 1 h. Rabies vaccine was administered intraperitoneally to all groups except the saline control group on day 0 and day 7. Mice were maintained and evaluated for up to 21 days after infection.

2.7. Phylogenetic analysis

Phylogenetic analysis was conducted by using MEGA 7.0 software. Complete G gene sequences of rabies strains were obtained from GenBank or collected from Wuhan Institute of Biological Products and the Military Veterinary Research Institute, Academy of Military Medical Sciences.

2.8. Ethics statement

All the animal experiments described in this study were conducted according to the Guidelines on the Human Treatment of Laboratory Animals stipulated by the Ministry of Science and Technology of the People's Republic of China. Procedures in this study were designed to avoid or minimize discomfort, distress, and pain in the animals.

3. Results

3.1. Identification of ormutivimab as a broad and potent inhibitor of rabies virus infection

In determining the value of an antiviral biologic, the neutralization breadth and potency are the two most crucial parameters. To analyze breadth of ormutivimab neutralization, we firstly investigated the distribution of rabies cases in China. As shown in Fig. S1, rabies cases in China were mostly concentrated in the southwest, eastern, and central regions by the end of 2017 [33,34]. From these endemic regions, we selected a broad panel of rabies strains of dog, bat, and Chinese ferret badger origins to determine the neutralization abilities of ormutivimab, although >95% of human rabies cases are caused by rabid dogs [33]. To understand the grouping and evolution characteristics of rabies virus used here, phylogenetic analysis was conducted using G gene complete sequences. Results showed that the isolates covered four mainly China rabies clades (Fig. S2), indicating the RABVs we selected are representative.

Ormutivimab neutralization ability was tested via FAVN, which was recommended by Office International des Epizooties and had no difference in sensitivity or specificity compared with Rapid Fluorescent Focus Inhibition Test assay [31]. The potency at 50% neutralization against the CVS-11 standard challenge strain was 1037.5 IU/mL shown in Table 1. For comparison, about 27.8% (5 in 18) of the isolates had similar end point titers and the same percentage of the isolates (5 in 18) had neutralizing potency >1300 IU/mL, indicating ormutivimab neutralized these isolates at a lower antibody dose compared with CVS-11 strain. There have also been 4 Chinese RABV strains whose end point titer was comparable to that of CVS-24 standard challenge strain. The titer for ormutivimab against GZ-3BF and Flury-LEP was 1:198 and 1:384.5 respectively, which 3- or 5-fold lower than the titer against the CVS-11 strain, demonstrating ormutivimab weakly but indeed neutralized these two rabies virus. The *in vitro* results indicated ormutivimab was able to neutralize a broad panel of Chinese prevalent street RABVs

Table 1

Ormutivimab neutralization activity and potency against prevalent Chinese RABV strains.

Strains	Location	Neutralizing potency (IU/mL)
JX08-45, Chinese ferret badger, Street RABV	Jiujiang City, Jiangxi Province	1323.8
BZ08, Dog, Street RABV	Sichuan Province	673.3
8202, Deer, Street RABV	Jilin Province	581
BD06, Dog, Street RABV	Baoding City, Hebei Province	673.3
HZ09, Dog, Street RABV	Hanzhong City, Shanxi Province	1093.5
YN01, Homo sapiens, Street RABV	Yunnan Province	1376.3
RY10-2, Dog, Street RABV	Raoyang City, Hebei Province	1056.6
JX12-234, Chinese ferret badger, Street RABV	Jiangxi Province	1428
JS07-21, Dog, Street RABV	Jiangsu Province	1356.1
GN07, Dog, Street RABV	Guangning City, Guangdong Province	963.5
GZ-3BF, Bat, Street RABV	Guizhou Province	198
aG, Vaccine strain, Dog	Beijing City	526.5
CTN, Vaccine strain, Dog	Shandong Province	963.5
ERA, Vaccine strain, Dog	USA	946.2
Flury-LEP, Vaccine strain	N/A	384.5
SRV ₉ , Vaccine strain	N/A	1487.6
CVS-24, Standard challenge strain	N/A	581
CVS-11, Standard challenge strain	N/A	1037.5

NOTE. Neutralizing potency was determined by Fluorescent Antibody Virus Neutralization (FAVN) Test. RABV, rabies virus; N/A, nonapplication.

at varying neutralization capacity.

3.2. Protective efficacy of ormutivimab in challenged KM mice against various rabies virus

To determine the protective activity of ormutivimab *in vivo*, groups of KM mice were challenged with BD06 street isolate, which is a well-characterized RABV in China and considered to be highly virulent [35]. Ormutivimab at a dose of 20 IU/kg and 100 IU/kg respectively were then administered. Numbers of surviving mice in each group were recorded daily up to 21 days post-injection. For comparison, mice injected with HRIG at doses of 20 IU/kg and 100 IU/kg were included as controls. The death started at day 8 in the saline-only control group, and all mice in this group died (0/10) within 15 days as shown in Fig. 1. Both ormutivimab and HRIG administration at 20 IU/kg led to 90% (9/10) survival, showing a marked survival benefit over the saline-only control group. In contrast, all mice were still alive (10/10) at the end of this experiment in groups treated with ormutivimab and HRIG at dose of 100 IU/kg. In line with the *in vitro* data, PEP with ormutivimab could prevent lethal rabies virus infection in a dose-dependent manner, and the protective activity of ormutivimab was comparable to that of HRIG.

Three more rabies virus strains were selected to further evaluate protective capability of ormutivimab in KM mice at a WHO-recommended dose of 20 IU/kg, as shown in Table 2. Mice were infected intramuscularly with 100 LD₅₀ of rabies viruses, including standard challenge strain CVS-24 and Chinese prevalent street isolates GZ-1 and GX-1 origins in dogs. After 4 h, either negative control saline or the immune globulins ormutivimab and HRIG was administered. The morbidity and mortality of mice were monitored for up to 21 days after infection. As expected, KM mice administered saline had all died. In the group challenged with strain GX-1, both HRIG- and ormutivimab-treated mice had a survival rate of 100% (10/10). Ormutivimab protected mice from rabies CVS-24 and GZ-1 isolates at a comparable rate to that of HRIG (90%). Taken together, these data reveal that the protection of ormutivimab against rabies virus *in vivo* are equivalent to that of HRIG.

3.3. Ormutivimab protects beagles from a lethal challenge of street RABV BD06

To further characterize the *in vivo* efficacy of ormutivimab, a PEP model was established using a total of 160 beagles. The dogs were infected intramuscularly with Chinese street RABV BD06.6 h later, the dogs received either ormutivimab (20 IU/kg) or HRIG (20 IU/kg) alone or together with vaccine. The first dose of the vaccine was administered immediately, and the other four doses were injected at day 3, 7, 14, and 28 after challenge. The morbidity and mortality of dogs were recorded daily for up to 90 days. All dogs in the challenge control group died at the end, as expected (Fig. 2), indicating a successful challenge with BD06. A comparable survival rate was observed in dogs administered ormutivimab only (96.7%) and HRIG only (100%). In contrast, vaccine alone could not provide adequate protection, showing a 60% (18/30) survival chance (Fig. 2). In this experiment, three dogs died of non-rabies as identified by direct fluorescent antibody test including two dogs in the group treated with ormutivimab and vaccine (hereafter ormutivimab + vac.) and one dog in the group injected HRIG plus vaccine (hereafter HRIG + vac.). No death due to rabies (28/28) was observed in the ormutivimab + vac. group, and the rate of survival from rabies in the HRIG + vac. group (positive control) was 100% (29/29). The data showed that ormutivimab plus vaccine provided a complete protection against BD06 infection in dogs.

3.4. Vaccine potency in challenged beagles treated with ormutivimab or HRIG

The inhibitory effect of antibody on active immunity induced by

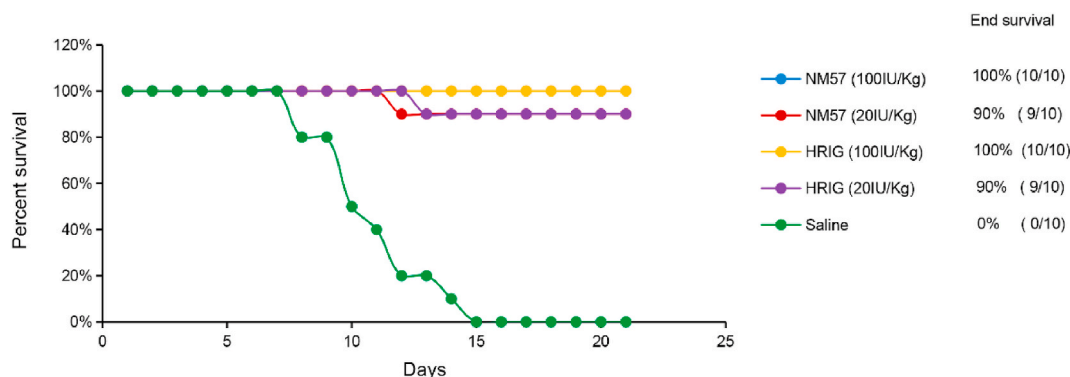


Fig. 1. Ormutivimab post-exposure protection in a Kunming mouse model. Groups of Kunming mice were injected with 100 MILD₅₀ of BD06 RABV. Four hours after infection, ormutivimab or HRIG at 100 IU/kg and 20 IU/kg was injected into the same site as the infection site. The morbidity and mortality of the mice were monitored for up to 21 days post-infection. Numbers of surviving mice for each group per day are plotted in this graph. Saline-treated groups were included as negative controls.

Table 2

Post-exposure protection effect of ormutivimab in KM mouse model challenged by CVS-24, GZ-1 and GX-1 strains.

Group	Challenge virus	Treatment	Survival	Percent Survival
1	CVS-24	Ormutivimab (20 IU/kg)	10/10	100%
2		HRIG (20 IU/kg)	9/10	90%
3		Saline	0/10	0%
4	GZ-1	Ormutivimab (20 IU/kg)	10/10	100%
5		HRIG (20 IU/kg)	9/10	90%
6		Saline	0/10	0%
7	GX-1	Ormutivimab (20 IU/kg)	10/10	100%
8		HRIG (20 IU/kg)	10/10	100%
9		Saline	1/10	0%

NOTE. CVS24 is a standard challenge strain. Street RABV strains GZ-1 and GX-1, isolated from dog, were kept at the Military Veterinary Research Institute in China.

vaccination against rabies was observed [36]. Therefore, it is critical to evaluate the interference degree. To determine the effect of ormutivimab on vaccine potency, we analyzed serum RVNA titers in surviving beagles in each treatment group at different time points after BD06 challenge (Table 3).

Serum RVNA titers were somewhat lower in beagles that received immune globulin plus vaccine than those received vaccine alone (Fig. 3), which was in agreement with previous studies [36]. The inhibition

degree induced by ormutivimab was lesser than that induced by HRIG in contrast. On day 3, serum RVNA titers could be measured in all three groups (Fig. 3). An RVNA titer 0.5 IU/mL, which is globally recognized as the threshold of seroconversion for humans, was observed from day 7 in the beagles treated with 20 IU/kg ormutivimab plus vaccine, suggesting that the beagles were protected sooner with ormutivimab than HRIG (Figs. 3, 4A and 4B). Because it will take approximately 7–10 days after initiation of vaccination to produce protection antibodies, rabies immune globulin administration during this period is very important. Serum RVNA titers continued to increase until day 28, with no significant difference between the three treatment groups (Figs. 3 and 4C), which because active immunization stimulates the host immune system to produce enough neutralization antibodies during this period. All beagles had serum RVNA titers above the accepted protection level for rabies (0.5 IU/mL) until day 90, when the experiment was terminated. Altogether, ormutivimab provided a faster protection and weaker vaccine interference, compared with HRIG (Table 3).

4. Discussion

Human rabies is a terrible disease that can be prevented by timely and appropriate PEP. However, it has been estimated that less than 10% of category III exposed patients receive RIGs due to high cost and low availability [37]. Thus there still have about 60,000 rabies death each year globally. Contamination risks and variation between batches are also issues of RIGs need to be addressed. Given the limitations, the new recommendations stipulate a more prudent use of RIG and now include rabies mAbs in its recommendations stating that, if available, the use of

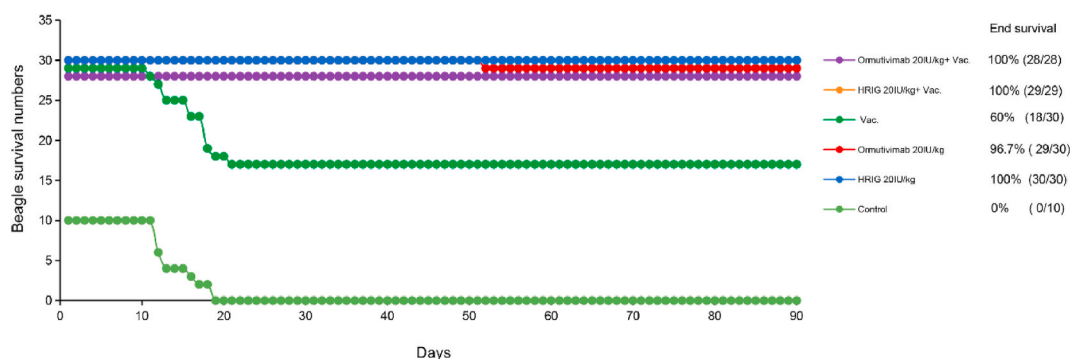


Fig. 2. Protective efficacy of ormutivimab against rabies in a beagle PEP model. Ormutivimab or HRIG at 20 IU/kg was administered in conjunction with rabies vaccine to RABV (BD06)-infected beagles at 6 h post-infection. The other three groups were treated only with vaccine or ormutivimab or HRIG, respectively. The challenge group was included as a control. Five doses of vaccine were administered on day 0, 3, 7, 14, and 28. Animal mortality and morbidity were monitored daily until 90 days after challenge. Numbers of surviving dog for each group per day were plotted in this graph.

Table 3

Serum rabies virus neutralizing antibody (RVNA) titers in challenged Beagles.

Treatment	Survival	Serum rabies virus neutralizing antibody (RVNA) titers in challenged Beagles (IU/mL)						
		0 d	3 d	7 d	14 d	28 d	60 d	90 d
Ormutivimab 20IU/kg + Vac.	28/28	0	0.31 ± 0.19	1.01 ± 0.79	10.35 ± 10.67	46.40 ± 11.39	13.93 ± 8.88	2.85 ± 1.60
HRIG 20 IU/kg + Vac.	29/29	0	0.34 ± 0.32	0.30 ± 0.20	2.97 ± 3.52	48.70 ± 8.18	18.99 ± 9.42	2.69 ± 0.98
Vaccine (Vac.)	18/30	0	0.06 ± 0.05	1.04 ± 0.84	16.82 ± 12.30	51.28 ± 4.80	22.06 ± 13.97	7.01 ± 9.15

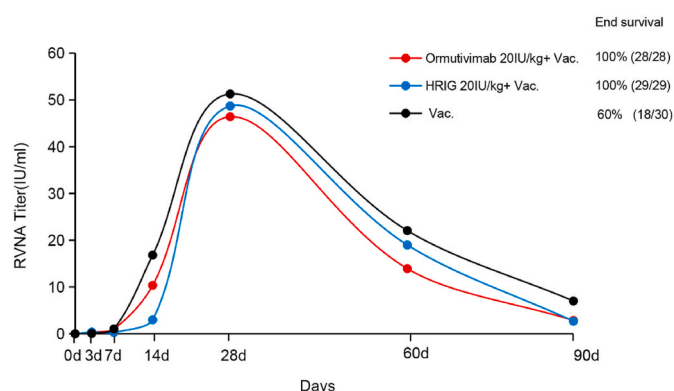


Fig. 3. Serum rabies virus-neutralizing antibody (RVNA) titers in challenged beagles. On the day of the challenge (day 0), beagles in each treatment group were treated with rabies vaccine, or vaccine with 20 IU/kg ormutivimab, or vaccine with 20 IU/kg HRIG as control. On days 0, 3, 7, 14, 28, 60, and 90 after treatment, serum was obtained from the surviving beagles and analyzed for RVNA titer via the fluorescent antibody virus neutralization test.

mAb products instead of RIG is encouraged [37]. Here, we reported a recombinant human IgG1 type monoclonal antibody ormutivimab, which binds the liner epitope I of the glycoprotein of rabies virus. Our results revealed that ormutivimab possesses broad-spectrum neutralization activities and high protective potency in PEP and could be an effective candidate for human rabies PEP.

In our study, we selected 18 Chinese prevalent RABV strains (Fig. S1) isolated from different hosts to evaluate the neutralizing capacity of ormutivimab via FAVN test. Although with varying neutralization strength, ormutivimab could inhibit the rabies viruses of dog, bat, and Chinese ferret badger origins listed in Table 1. These RABV strains were distributed in the endemic regions in China (Fig. S1). Chinese rabies street strains can be classified into six clades (Clade I–VI), and only two main lineages, Clade I and Clade II, contributed to the rabies epidemic in mainland China [38]. The rabies viruses used in this study belonged to

Clade I–IV (Fig. S2), indicating that ormutivimab had a broad-spectrum protection capacity against at least Clade I–IV rabies viruses in China. In addition, given that ormutivimab shares the same code sequence with anti-rabies antibody SO57 [15] and CR57 [16,22,39], their breadth neutralization activities also indicated ormutivimab is one of the most potent anti-rabies antibody.

To estimate its protection potency *in vivo*, three different animal models for PEP were established. Ormutivimab was firstly investigated at a higher dose in a PEP model in Balb/c mice against the Chinese prevalence street isolate *Jian*. The survival rate in mice receiving ormutivimab at 200 IU/kg dose plus vaccine reached 90%, which is comparable to that achieved HRIG (100%) (Table S1). Kunming mice were used as the second animal model, in which a highly virulent, well-characterized RABV BD06 street strain was selected to challenge. Ormutivimab was shown to have equivalent protection to HRIG at doses of 20 IU/kg and 100 IU/kg, resulting in 90% (9/10) and 100% (10/10) survival rate respectively (Fig. 1). In addition, ormutivimab alone at 20 IU/kg provided significant benefits to survival (100%) (Table 2) against CVS-24, GZ-1 and GX-1 strains. For comparison, results revealed that Rabishield alone protected 100% of the hamsters challenged with Texas coyote rabies virus isolate with at least 7 IU/kg doses. We didn't-test the minimum dose of ormutivimab for 100% protection in mice. The efficacy of ormutivimab in a PEP model in dogs infected with BD06 RABV was further tested. As shown in Fig. 2, ormutivimab at 20 IU/kg in combination with vaccine or alone resulted in 100% and 96.7% survival, respectively. SYN023, an anti-rabies monoclonal antibody cocktail, at 0.3 mg/kg dose together with vaccine yielded 90% survivorship of BD06 rabies virus challenged hamsters [24]. Basis on our unpublished data, ormutivimab at 20 IU/kg almost equal to 0.025 mg/kg, which is ten-fold lower than the dose of SYN023 for achieving 90%–100% protection. The launched product Rabishield at 21 IU/kg dose plus with vaccine protected 95% of hamsters inoculated with Texas coyote rabies virus isolate [25]. Our results indicate that the efficiency of ormutivimab is as well as the other advanced rabies mAbs.

Higher doses of RIGs have been shown to reduce vaccine function during PEP [40–46]. Therefore, the degree of vaccine interference is another important consideration besides protection efficacy. In the

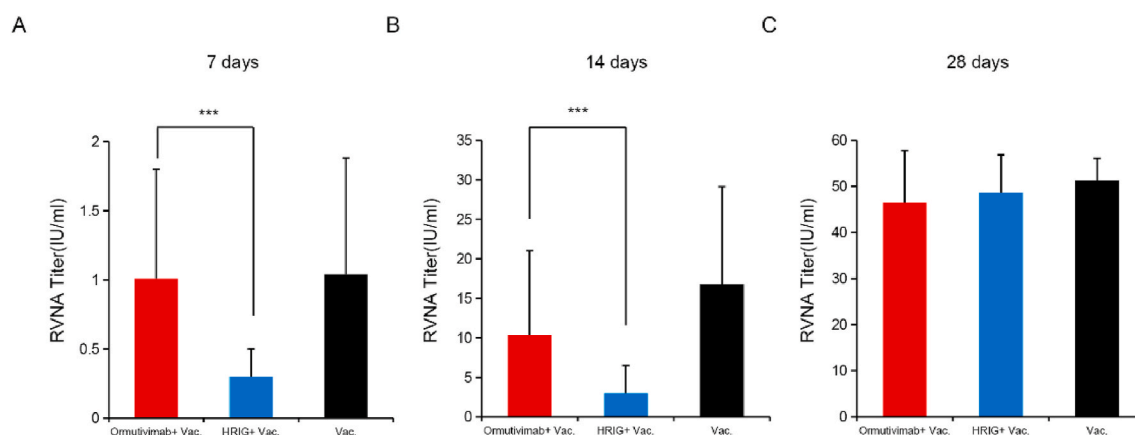


Fig. 4. Serum rabies virus-neutralizing antibody (RVNA) titers in challenged beagles on days 7, 14, and 28. The bar graphs show RVNA titers on days 7 (A), 14 (B), and 28 (C). Differences between groups were tested using unpaired *t*-test. *** indicates $P < 0.001$, and the bars represent.

present study, we examined the potential vaccine interference effect of ormutivimab at 20 IU/kg in virus-challenged beagles. RVNA titers were monitored at a specified time within 90 days (Table 3). Some interference was observed in RVNA profiles both in the 20 IU/kg ormutivimab and 20 IU/kg HRIG groups (Fig. 3), which was consistent with the results of previous studies [47]. Interestingly, RVNA titers on day 7 in the ormutivimab group (1.01 ± 0.79 IU/mL) were already higher than 0.5 IU/mL (Figs. 3 and 4), which is considered indicative of an adequate immune response to vaccination. Importantly, from day 7 to day 14, significantly higher RVNA titers was observed in the ormutivimab treated group (Figs. 3 and 4). RVNA titers in all three groups were still maintained at above 0.5 IU/mL till day 90. These data suggested the degree of vaccine interference induced by ormutivimab was slightly lower than HRIG.

In conclusion, with *in vitro* and *in vivo* experiments, ormutivimab exhibited a broad-spectrum protective efficacy against Chinese prevalent RABV strains and showed equivalence to HRIG with respect to survival in PEP models and level of vaccine interference. Finally, we envision that safer and effective RIG alternatives, such as ormutivimab, with reduced production cost and steady supply, would be effective in rabies PEP, particularly in endemic areas.

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Author contributions

Li-li zhai, Writing, Data curation, Visualization, and Software. Hui Wang, Resources and Investigation. Wei Zhao, Formal analysis and Investigation. Shou-feng Zhang, Methodology, Validation, Supervision and Project administration. Fa-ming Miao, Formal analysis, Investigation, and Supervision. Yang Cao, Software and Visualization. Chen Chen, Software and Investigation. Yu-Feng Li, Investigation and Resources. Jie Gao, Investigation. Ruo-yun Lv, Investigation. Shi-xiong Zhang, Investigation. Jia-bin Cao, Investigation. Xu-fan Zhang, Investigation. Ming-ming Yang, Investigation. Bin Zhang, Investigation. Jing Zhao, Investigation. Jing-shuang Wei, Conceptualization, Methodology, Validation, Project administration, and Funding acquisition. Jian GAO, Conceptualization and Funding acquisition.

Declaration of competing interestDoCI

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement mentioning any meeting(s) where the information has previously been presented: The study presented in this article has not previously been presented elsewhere.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tmaid.2022.102267>.

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