

REVIEW

OPEN ACCESS



Advances in the progress of monoclonal antibodies for rabies

Linlin Fan^a, Li Zhang^b, Jingxin Li^{a,b,c}, and Fengcai Zhu^{a,c,d}

^aCenter for Global Health, School of Public Health, Nanjing Medical University, Nanjing, PR China; ^bVaccine Clinical Evaluation Department, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, PR China; ^cInstitute of Global Public Health and Emergency Pharmacy, China Pharmaceutical University, Nanjing, PR China; ^dNHC Key Laboratory of Enteric Pathogenic Microbiology, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, PR China

ABSTRACT

Rabies is a highly fatal zoonotic disease caused by the rabies virus invading the central nervous system. When suspected of exposure to the rabies virus, post-exposure prophylaxis should be administered as soon as possible. Monoclonal antibodies (mAbs) neutralizing the rabies virus could be better in human rabies post-exposure prophylaxis than equine or human rabies immune globulin in terms of supply, cost, and efficacy. This article reviews anti-rabies mAbs produced by multiple techniques, and the results of clinical trials for anti-rabies mAbs cocktails recognizing non-overlapping epitopes are also discussed.

ARTICLE HISTORY

Received 10 November 2021
Revised 6 December 2021
Accepted 22 December 2021

KEYWORDS

Rabies virus; glycoprotein; monoclonal antibody; antibody library; antibody cocktail

Introduction

Rabies virus (RABV), a single-stranded RNA virus of the genus *Lyssavirus*, family *Rhabdoviridae*, invades the central nervous system, causing acute and zoonotic natural foci disease worldwide.¹ RABV was rod-shaped or bullet-shaped with a diameter of about 75 nm and a length of 100–300 nm under an electron microscope, which Louis Pasteur first isolated in 1885. The virus genomic RNA encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and polymerase protein (L). Glycoproteins are the major surface antigens to which neutralizing antibodies are bound. Glycoprotein antigen binds to acetylcholine receptors, leading to neurotransmitter release disorder and causing neurological symptoms.^{2,3} The glycoprotein molecule consists of three domains: an ectodomain (amino acid, a.a.1–439), a transmembrane domain (a.a.440–461), and a cytoplasmic domain (a.a.462–505). RABV glycoprotein has distinct antigenic sites (AS): I (a.a.226–231), II (IIa a.a.198–200, IIb a.a.34–42), III (a.a.330–338), IV (a single a.a.251), minor site a (a.a.342–343, otherwise referred to as G1), and G5 (a.a.261–264, also comprises AS VI, a single a.a.264).^{4,5} The positions of AS of the glycoprotein are shown in Figure 1. The vast majority of mAbs recognizes AS I and III of rabies glycoprotein.⁶ The literature has not reported the mAb candidates bound to the minor site a, AS IV, and AS VI.

RABV enters the human body through bites, scratches, or licks on damaged skin and mucous membranes of infected animals. Dogs, wolves, and cats are the main reservoirs and play a significant role in the process of carrying and spreading the virus.⁷ As a result of infected-animal bites, the virus moves from peripheral nerve endings to the spinal cord, then finally penetrates the brain. The incubation period of rabies is 1–

3 months on average, mostly around 5 days to 1 week, rarely more than 1 year. The incubation period of rabies is related to the bite site. The closer the bite site is to the central nervous system and brain, the shorter the incubation period. Infected dog bites cause almost all cases of infection in humans. Effective dog and wildlife vaccines are critical in the reduction and even elimination of rabies in developed countries. However, the complexity of the animal-human interface of the virus makes elimination challenging. It requires significant investment in the administration of animal vaccination and efforts to reduce rabies virus infection. Despite successful control worldwide, rabies continues to result in tens of thousands of fatal cases each year, especially in Africa and Asia, where children under the age of 15 accounts for 40% of the fatal cases.⁸ There is an urgent need to ensure that suspected rabies exposures receive immediate treatment to prevent almost fatal clinical rabies.

Rabies post-exposure prophylaxis in human

The World Health Organization (WHO) has developed specific recommendations for rabies post-exposure prophylaxis (PEP) in humans. There are three categories of exposure to suspected rabid animals. Category I exposures only touch or feed animals that lick on intact skin. Category II exposure is defined as nibbling of uncovered skin, minor scratches, or abrasions without bleeding. Category III exposure involves single or multiple transdermal bites or scratches, contamination of mucous membranes or broken skin by saliva from animal licks, and exposure due to direct contact with bats.⁹ Thorough wound cleaning should be immediately administered for category I and category II exposures. Category II

CONTACT Jingxin Li ✉ jingxin42102209@126.com Vaccine Clinical Evaluation Department, Jiangsu Provincial Center for Disease Control and Prevention, No. 172 Jiangsu Road, Gulou District, Nanjing City, Jiangsu Province, China; Fengcai Zhu ✉ jszfc@vip.sina.com Center for Global Health, School of Public Health, Nanjing Medical University, No. 101 Longmian Avenue, Jiangning District, Nanjing City, Jiangsu Province, China

© 2022 The Author(s). Published with license by Taylor & Francis Group, LLC.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

exposures should administer the rabies vaccine as soon as possible. Administration of rabies immunoglobulin (RIG) for wounds classified as category III exposure, is of utmost importance in wound management. RIG should be infiltrated around the wound as much as anatomically feasible and remaining RIG should be injected at an intramuscular site distant from that of vaccine inoculation. It takes some time for active immunity to be triggered by vaccines; thus, passive immunization with RIG can neutralize the rabies virus, while the active immune response develops. The rabies vaccine and RIG combination can almost wholly prevent disease development as soon as possible.¹⁰ It is estimated that nearly 29.2 million people worldwide undergo rabies PEP regimen annually,¹¹ but only a tiny part of category III exposures actually receive RIG, which is a lifesaving treatment.

Currently, rabies RIGs used in treatment are mainly related to equine rabies immune globulin (ERIG) and human rabies immune globulin (HRIG).^{12,13} ERIG derived from the plasma of immunized horses may cause serious side effects and hypersensitivity reactions.^{14,15} Production was largely discontinued due to animal protection groups. HRIG derived from the serum of immunized humans is considered safer than ERIG, but it is hindered by its high cost and the insufficiency of donors.

Since rabies vaccines cannot provide comprehensive and timely protection, RIG is limited due to supply, cost, and safety. Rabies mAbs can be the best choice in rabies PEP. It has broad application prospects with the advantages of high neutralizing activity, strong specificity to the corresponding target, and easy to standardized production, and so on.¹⁶ Compared with polyclonal RIG (ERIG and HRIG) bound to different rabies virus epitopes, single mAb interacts with only one viral epitope. Thus, the ideal monoclonal antibody preparation used in urgent PEP should also meet the following requirements. First, the preparation must contain at least two different antibodies bound to nonoverlapping epitopes to neutralize the virus

broadly. Second, the preparation must be safe, and the efficiency is not inferior to the existing HRIG. Last but not least, the cost of preparation must be acceptable for patients needing PEP, especially those in underdeveloped countries where fatal cases are most common.

Current licensed RABV mAbs

There are currently two licensed anti-rabies monoclonal antibodies in India, and three candidates have reached clinical trials (Table 1). The recombinant anti-rabies mAb preparation Rabishield (SII RMAb)³² was licensed in India in December 2016; The mouse-derived monoclonal antibody preparation TwinrabTM (RabiMabs)³³ developed by Crucell in the Netherlands and Zydus Research Center was licensed in India in September 2019.

Rabishield (SII RMAb)

The recombinant anti-rabies mAb Rabishield (SII RMAb) is a human IgG monoclonal antibody that binds to a conformational epitope of rabies glycoprotein, including antigenic site III. SII RMAb was developed by MassBiologics (Boston, Massachusetts, USA) under the name 17C7, then further developed under the name SII RMAb. 17C7 derived initially from transgenic mice carrying human immunoglobulin genes. 17C7 was the most broadly neutralizing mAbs, neutralizing 92% of the 25 rabies virus isolates tested. In the hamster challenge experiment, SII RMAb showed a higher neutralizing effect than ERIG or HRIG and did not interfere with the immune response of the rabies vaccine in the standard PEP regimen.^{34,35} These preclinical data support the initiation of clinical trials to demonstrate safety in humans. Gogtay et al.¹⁷ conducted a randomized, open-label, dose-escalating (1, 3, 10, and 20 IU/kg) phase 1 clinical trial in healthy adults in India. SII RMAb was well tolerated during

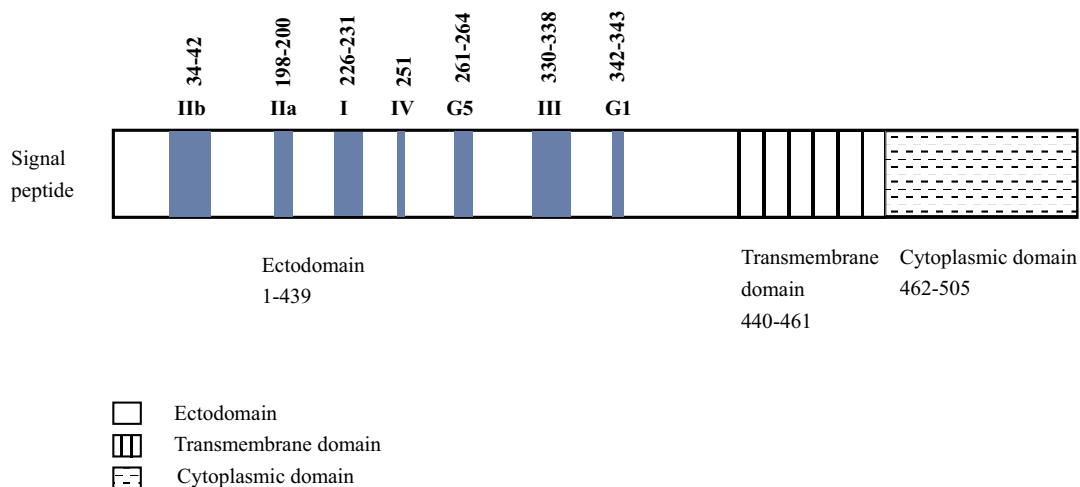


Figure 1. Antigenic sites of RABV glycoprotein. Numerals represent amino acid residue numbers.

Table 1. Rabies mAbs licensed or in clinical development.

Name	Developer	Stage	Clinical trials registry number
Licensed			
Rabishield (SII RMAb)	Serum Institute of India PVT. LTD. (SIPL)	Phase 1, 2/3 clinical trials completed and licensed in December 2016	CTRI/2009/091/000465 ¹⁷ CTRI/2012/05/002709 ¹⁸
Twinrab™ (RabiMabs)	Zydus Cadila in India	Phase 1/2, 3 clinical trials completed and licensed in September 2019	CTRI/2015/06/005838 ¹⁹ CTRI/2017/07/009038 ²⁰
Applying for licensure			
rhRIG	NCPC (China) in collaboration with MITT (USA)	Phase 1, 2 and 3 clinical trials completed	NCT02559921 ²¹ ChiCTR1900023809 ²² ChiCTR1900023785 ²³ ChiCTR1900023236 ²⁴ ChiCTR1900021478 ²⁵
In active clinical development			
SYN023 (CTB011/CTB012)	Synmore Biologics in China	Phase 1 and 2 clinical trials completed and phase 3 clinical trials are planning	CTR20190281 ²⁶ NCT02956746 ²⁷ NCT04644484 ²⁸
Withdrawn from development			
CL184 (CR57/CR4098)	Crucell in Netherlands	Phase 1 and 2 clinical trials completed	ISRCTN18660493 ²⁹ ISRCTN12693237 ²⁹ NCT00708084 ³⁰ NCT01228383 ³¹

the study. The geometric mean concentration (GMC) of rabies neutralizing antibodies was comparable to that of 20 IU/kg of HRIG when SII RMAb at a dose of 10 IU/kg, and both cohorts combined with the vaccine (Rabivax™, India). The GMC of rabies neutralizing antibody was 23.4 IU/ml vs. 15.3 IU/ml on day 14 evaluated by the rapid fluorescence focusing inhibition test (RFFIT), respectively, in the vaccine plus SII RMAb cohort and in the vaccine plus HRIG cohort. Later, they conducted a phase two-thirds randomized, single-blind, non-inferiority study among 200 participants with suspected rabies category III exposure. Participants received SII RMAb or HRIG (Imogam, Sanofi Pasteur) randomly in a ratio of 1:1. They all cleaned the wounds thoroughly and administered five doses of rabies vaccine (Rabivax™, India) intramuscularly (0, 3, 7, 14, and 28 days). The GMC ratio of neutralizing antibodies in the SII RMAb group to the HRIG group was 4.23 (96.9018% confidence interval [CI], 2.59–6.94) on the 14th day, with a GMC of 24.90 IU/mL for the SII RMAb group and 5.88 IU/mL for the HRIG group. SII RMAb was superior to HRIG rabies virus-neutralizing activity (RVNA) levels in suspected rabies category III exposures.¹⁸ A post-marketing safety study conducted in Bangalore included 397 category III exposures (142 in the SII RMAb group, 243 in the ERIG group, and 12 in the HRIG group). All patients were treated according to the PEP regimen as recommended. About 8% subjects in the SII RMAb group had delayed adverse reactions such as pain (4.2%), swelling (2.1%), and wound infection (0.7%) at the infiltration site, without any complications. There was no significant difference in safety evaluation among the three groups. The new human mAb was safe from post-exposure prophylaxis against rabies.³⁶ There were potential concerns about the product. It

contains only one monoclonal antibody shown to be unable to neutralize a rare rabies variant found in Peruvian bats in the Americas region.^{18,37} The WHO working group considered that as it was a first-licensed product and limited use of RIG, using it in specific areas and conducting post-marketing surveillance could help solve the above concern. They also figured that SII RMAb was worth learning for future production and expected to be an effective alternative to RIG, especially in countries where most rabies cases occur.

Twinrab™ (RabiMabs)

This product combines two mouse monoclonal antibodies (M777-16-3 and 62-71-3) that bind to AS II and III of rabies glycoprotein, respectively. The two mAbs were donated by the WHO Collaborating Centers for Rabies (M777-16-3 from the Animal Diseases Research Institute, Canada, and 62-71-3 from the Centers of Disease Control and Prevention, USA). The binding of RabiMabs to two distinct antigenic sites provides adequate protection against a mutated rabies virus due to a mutation. The equipotent combination of 62-71-3 and M777-16-3 was efficacious in hamsters challenged with a lethal dose of rabies virus.³⁸ Phases 1/2 and 3 clinical trials completed are shown on the Indian Clinical Trials Registry website. In the randomized, double-blind, placebo-controlled phase 1/2 study, RabiMabs was safe and well tolerated up to a dose of 40 IU/kg in conjunction with the rabies vaccine (Vaxirab N, Zydus Cadila).¹⁹ In the phase 3 trial, a total of 308 category III suspected rabies exposures were randomly assigned to RabiMabs (40 IU/kg) group and HRIG (20IU/kg) group in a 1:1 ratio. All exposures received wound cleaning and rabies vaccination (Vaxirab N, Zydus Cadila). The rabies virus neutralizing antibodies (RVNA) in the clinical samples was determined by RFFIT. This study confirmed that Twinrab™ was safe and non-inferior to HRIG, and that it could provide protection up to day 84 in combination with the rabies vaccine.²⁰ This study was conducted in subjects with suspected rabies exposure rather than a confirmed infection. In other words, there was no evidence to prove rabies in the brain tissue of infected dogs.

Rabies mAbs obtained by different methods

Hybridoma technique

The hybridoma technology made mAbs possible to obtain, laying a theoretical foundation for future research. A somatic hybrid (hybridoma) between mouse myeloma cells immunized with rabies vaccine and spleen cells derived from BALB/c mice was produced by hybridoma technology in 1978. Hybridomas obtained by Wiktor et al.³⁹ was able to protect experimental animals from lethal rabies virus infection. Muhamuda⁴⁰ fused SP2/0 cells with spleen cells from BALB/C mice immunized with the rabies vaccine (Verorab, France) to obtain mAbs. The obtained mAbs was 2000 times more potent than commercial ERIG in terms of effective protein concentration and neutralizing titer. Cheng Wei⁴¹ fused the spleen cells of BALB/c mice immunized with the vaccine (Fort Dodge, America) with SP2/0 myeloma cells. Two anti-RABV mAb were obtained, named

1G8 and 4G11. Both mAbs showed high neutralizing activity by indirect immunofluorescence assay (IFA). The two mAbs recognized non-overlapping epitopes were tested by the convenient enzyme-linked immunosorbent assay (ELISA) with an additivity index of 16.8%.

The murine mAbs had limitations on instability and inability to provide adequate protection owing to species differences. Human hybridomas was prepared from peripheral B lymphocytes from vaccines with the human rabies vaccine fused with somatic cells. One of the nine human mAbs, SO57, neutralizing a variety of RABV strains, was described by Dietzschold et al.⁴² NM57 is expressed and purified from a stable recombinant expression system in CHO cells. This is the prototype of rhRIG, a Chinese monoclonal antibody introduced later.⁴³ Bakker et al.⁴⁴ reformatted SO57 (renamed CR57) into their expression system for production in PER.C6 cells. CR57 showed high neutralizing potency in vitro and in vivo and recognized AS I by characterizing CR57 escape mutants.⁴⁵ The above mAbs provide candidate antibodies for monoclonal antibody cocktails.

Antibody library technique

Antibody library technology aims to obtain mini-antibodies (single-chain antibody fragment, scFv) or Fab fragments of human antibodies through affinity screening from combinatorial libraries.⁴⁶ We then construct full-size human immunoglobulins based on selected fragments.⁴⁷ Phage and ribosomal display of combinatorial libraries are widely used to screen antibody fragments nowadays.

Phage display of combinatorial library technique

Kramer et al.⁴⁸ isolated lymphocytes from the blood of rabies vaccinated volunteers conduct RABV phage display antibody libraries. The antibodies were selected using the inactivated virus and purified RABV glycoprotein. CR4098 was the best candidate bound to the AS III. Zhao et al.⁴⁹ constructed a human phage antibody libraries of 7×10^8 clones. They successfully screened a new rabies mAb, named scFv-S12, bound explicitly to the rabies virus and had high affinity. A novel human single chain variable fragment of mAb (AR16) was obtained from a phage display library with a repertoire of approximately 10^8 clones, and AR16 recognized the G5 epitope of RABV specifically.⁵⁰ In addition, a combinatorial scFv antibody phage library was constructed using a pttAL14 vector from the peripheral blood of immunized volunteers. Li Yu⁵¹ selected six mAbs with high affinity represented by high-level neutralizing activity using RFFIT. Fang and her team constructed a human scFv phage antibody library of 8.3×10^8 clones. They successfully verified 20 humanized anti-rabies mAbs with a neutralizing activity of more than 500 IU/mg.⁵²

The Human Fab Library was constructed from peripheral blood lymphocytes of rabies vaccinated donors with 2×10^7 clones. The three selected mAbs could specifically bind to antigenic site III and have the ability to neutralize CVS-11 strain (challenge virus standard, GenBank number accession GQ918139.1). Purified Fab antibody fragments were

considered more stable and had higher affinity than mini-antibodies (scFv).⁵³ Sun et al.⁵⁴ established a human phage Fab antibody library derived from vaccinated human donors. A panel of 11 mAbs specific for RABV glycoproteins were selected; five of them (RV01, RV03, RV05, RV08, and RV09) revealed high binding affinity and neutralizing activities by RFFIT in vitro. Epitope mapping and binding competition analysis showed that five human neutralizing antibodies recognized the antigenic site II of RABV glycoprotein.

Ribosomal display of combinatorial library technique

The scFv gene library was converted for phage display (constructed from the peripheral blood lymphocytes of three immunized donors described before)⁴⁹ to ribosome display format by polymerase chain reaction (PCR). The mAbs was obtained using purified PCR products for in vitro transcription and translation after five selection rounds. These mAbs with high affinity showed binding activity to the rabies virus.⁵⁵ Compared with phage display technology, the outstanding advantage of ribosome display technology is that the whole process of protein display and screening is performed entirely in vitro by a cell-free system, which overcomes the lack of cell dependence.^{56,57}

B cells immortalization technique

Human immune B cells from vaccines selected for the presence of serum antibodies capable of broadly neutralizing RABV were immortalized by transformation with the Epstein-Barr virus. Culture supernatants neutralizing the CVS-11 strain were tested to select mAbs. It has become the prototype of antibody RVC-20⁵⁸ and RVC-58 bound to AS I and AS III, respectively. Moreover, the combination of RVC20 and RVC58 protected hamsters from lethal RABV infection. The cocktail had the ability to neutralize all the RABV tested within a narrow range of concentrations compared with CR4098 and CR57.¹⁶ The characteristics of the above methods are shown in Table 2.

Rabies mAbs in clinical development

rhRIG

There are currently no rabies mAb licensed in China. Only recombinant human rabies immunoglobulin (rhRIG) is seeking licensure. The candidate consists of a mixture of human anti-rabies mAbs initially developed by Dr. Bernhard Dietzschold at Thomas Jefferson University. The North China Pharmaceutical Group Corporation (NCPG) has optimized the cell lines and processes for clinical material production. rhRIG could neutralize a wide range of rabies virus strains and provide protection in animal efficacy models.⁵⁹ Limited information about the product could only be available on the MTTI website with the Google search. Information on the Clinical trials.gov website showed that a phase 2 clinical trial that evaluated the rabies virus neutralizing activity, safety, and tolerability of rhRIG vs. HRIG in combination with human rabies vaccine in 300 healthy adults has been completed.²¹ After conducting a targeted search at the Chinese Clinical

Table 2. The characteristics of different methods to obtain monoclonal antibodies.

Methods	Advantages	Disadvantages
Hybridoma technique	Long history and mature technology; Operation process visualization.	Long production time; Antibodies need to be humanized; Unstable and poor reproducibility of hybridoma cells.
Antibody library technique	Low cost; Stable and strong specificity; Short production time and highly productive; Easy to humanize and restructure antibody structure.	High technical requirements; Limited by the capacity of the antibody library; If library mutations, it may be immunogenic to humans.
B cells immortalization technique	Small workload and short screening cycle; Independent of viral infection and genetic modification in vitro culture.	Insufficient numbers of B cells and antibodies; Short survival time and a limited extent in vitro culture.

Trial Registry (ChiCTR) website, two phase 1, one phase 2, and one phase 3 clinical trials were completed in Chinese subjects.^{22–25} Only one literature report found that a single injection with three doses (10, 20, 40IU/kg) of rhRIG was well tolerated in 48 healthy adults.⁴³

SYN023

Among the currently known candidate antibodies for the alternative of RIG, we should first mention the SYN023. Murine hybridomas (3D11E3 and 7G11A3) was generated by the fusion of splenocytes with P3X63Ag8.653 mouse myeloma cells followed by humanized by CDR grafting, human germline sequences homologous to the variable regions of 3D11E3 and 7G11A3 were chosen as the acceptor for humanization. Humanized 3D11E3 and 7G11A3 were then transfected with CHO DG44 cells to obtain CTB011 and CTB012. The humanized mAbs consists of a high percentage of the human immunoglobulin, and only complementarity-determining regions (CDRs) are taken from the mouse antibody. A new humanized monoclonal antibody cocktail mixture SYN023, composed of CTB011 and CTB012 mixed 1:1. SYN023 showed the ability neutralize rabies isolated from China and North America and offered the same protection as HRIG (20 IU/kg) in the Syrian hamsters challenged model.⁶⁰ They further assessed the efficacy of SYN023 in animal models, SYN023 afforded protection equal to standard HRIG (20 IU/kg) at 0.03 mg/kg in Syrian hamsters and 0.1 mg/kg in beagles. SYN023 at a dose of more than 0.1 mg/kg could reduce the neutralizing antibody titer similar to HRIG.⁶¹

An open, parallel, single-dose phase 1 study of healthy Chinese participants evaluated the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of SYN023 when administered alone or in combination with the rabies vaccine (lot number: 201804088, Chengda Biotechnology). All of the adverse events were grade 1 or 2, transient, and self-limited without long-term sequelae. The participants achieved protective levels of the rabies virus neutralizing antibody (≥ 0.5 IU/mL) on day 3 after injection SYN023. Characterization of the PK in Chinese indicated that SYN023 displayed a typical and

reliable PK, and SYN023 had similar PK/PD relationships in both Chinese and American participants.⁶² The American phase 2 trial compared the safety, PK and PD of a single dose of SYN023 and licensed rabies vaccines (Imovax, Sanofi Pasteur, RabAvert, GlaxoSmithKline) with one dose of HRIG (HyperRAB ST, Grifols) and licensed rabies vaccines (Imovax and RabAvert).²⁶ SYN023 at dosages of 0.3 mg/kg was considered to be relatively safe, effective and had good PK/PD profiles. Another study compared the safety and neutralizing activity of SYN023 intramuscularly (0.3, 1.0, 2.0 mg/kg) and subcutaneously (0.3 mg/kg) administration in healthy adults. There was no significant difference in adverse event frequency or profile with increased dose or with administration route. At a dosage of 0.3 mg/kg by either the IM or SC route, RVNA levels exceeded the generally accepted protective concentration (≥ 0.5 IU/mL) by day 1 after injection.²⁷ China is planning to start a phase 3 clinical trial on suspected rabies exposures.²⁸

CL184

CL184 cocktail was a 1:1 protein mixture of two human mAbs (CR57/CR4098) described before.^{44,48} The hamster model was used to evaluate the ability of CL184 to prevent fatal infections with four selected distinct bat RABV variants: silver-haired bat (Ln RABV); western canyon bat (Ph RABV); big brown bat (Efw1 RABV), and Mexican free-tailed bat RABV (Tb RABV). It showed that 42–100% of the animals survived when CL184 was administered with the rabies vaccine. By comparison, 19–95% survivorship against bat RABV variants was observed when HRIG (20 IU/kg) was combined with rabies vaccine. CL184 represented an efficacious alternative for HRIG in this study. We think CL184 and HRIG should be tested separately without vaccine to see how early protective levels appear in blood.⁶³ Bakker et al.²⁹ presented the first clinical data for CL184. When CL184 was administered in conjunction with the rabies vaccine (RabipurTM, Lot No. 1415), less than 40% of the participants had pain at the injection site, no fever, local induration, redness, or swelling. The RVNA levels of all participants were higher than the standard level (0.5 IU/mL) from day 14 in combination with the rabies vaccine. CL184 can be used in lower volumes than HRIG or ERIG due to its high concentration, contributing to better local tolerability. Phase 2 clinical trials have been completed in adolescents and healthy adults,^{30,31} but no published results of this trial could be found yet. Crucell was acquired by Janssen Pharmaceutical Companies of Johnson & Johnson in 2011, and the product is no longer listed to be in development on its website.⁶⁴

Discussion

Rabies mAbs can be obtained from large-scale culture directly after hybridization, and it is proved that hybridoma antibodies can protect animals against rabies virus attack. This is a milestone breakthrough in the development of rabies mAbs. The first generation of mAbs obtained by murine hybridoma had the same disadvantage as ERIG—the human anti-mouse antibody (HAMA) response. Due to species differences, the instability of some mouse hybridomas and the inability of animal mAbs to adequately

activate protective immune functions in the human organism. Humanized murine hybridomas by CDR grafting and human hybridomas come into being. Chimeric and humanized antibodies have become more and more a thing of the past, which were replaced by human antibodies. The phage antibody library technology provides new ideas for obtaining human neutralizing antibodies. Phage displays allow the exposure of peptides and proteins to the surface of filamentous phage, which is eventually selected as antibodies with high affinity and strong specificity. This modern method is highly productive, but if the library mutations, it may be immunogenic to humans. In addition, other technologies such as B cells immortalization, have broad application prospects in the future. One of the best broadly neutralizing mAbs RVC20 was shown to neutralize all 35 tested RABV strains across the world. No matter, which method is used to prepare mAbs, only when mAbs are used in combination to recognize non-overlapping epitopes can they exert the broadest neutralizing efficiency. The application of human mAbs cocktail binding to non-overlapping epitopes of the glycoprotein has been confirmed to neutralize standard rabies strain in hamster challenge models. What's more, the antibody cocktail must have a neutralizing effect on a broad range of natural RABV variants in the therapeutic antibodies used region.^{16,34,35} Lina Sun's team developed a cocktail composed of CR57⁴⁴ bound to AS I, RV08⁵⁴ bound to AS II, and RV3A5⁶⁵ (improved CR4098 by replacing the light and heavy chains) bound to AS III. The cocktail could neutralize the CVS-11 strain and 10 rabies virus street strains. The preparation provided better protection with a survival rate of 100% against RABV and also protected all hamsters when combined with the vaccine against RABV.⁶⁶ Further clinical trials should be conducted to determine the safety and effectiveness of the cocktail.

Despite rabies mAbs have been developing at home and abroad, fast clinical progress is challenging. Several of these mAbs have reached clinical trials with only two products gaining approval in India. Rabies mAbs was demonstrated to neutralize various rabies isolates in Syrian hamster challenge models, and other animal studies should also be conducted in preclinical studies. Early clinical studies in humans are relatively uncomplicated. The phase 1 study is main to explore the appropriate dose and safety evaluation. In phase 2 studies, pharmacokinetics, half-lives, neutralizing activity, and vaccine interaction/inhibition should be evaluated and compared with RIG. The sample size hindered the efficacy evaluation of rabies mAbs in phase 3 studies with category III exposures. That is, if dog bite incidence is low, it may take a long time to enroll adequate exposures to generate sufficient statistical power. The cost of production and conducting a trial in a large sample size, post-marketing surveillance may be too high to ensure return on investment for developers. Besides, all the phase 3 studies were conducted in category III suspected rabies exposures. The outcome of the suspected exposures should continue to be tracked to further determine the efficacy of the rabies mAbs. The efficacy of mAbs should be further confirmed in patients bitten by lab confirmed animals.

Conclusion

From serum polyclonal antibodies to hybridoma mAbs and from murine mAbs to human mAbs, considerable progress has been made in the urgent post-exposure prophylaxis of rabies. The efficiency of neutralizing mAbs cocktails has been confirmed in clinical trials. Developing a neutralizing rabies cocktail binding to the nonoverlapping epitopes of the glycoprotein is a grand challenge, and it also points out the direction for subsequent research. Safe, effective, and affordable rabies mAbs taking the place of HRIG in rabies PEP is just a matter of time.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported that there is no funding associated with the work featured in this article.

Abbreviations

RABV	Rabies virus
mAbs	Monoclonal antibodies
a.a.	Amino acid
AS	Antigenic sites
WHO	World Health Organization
PEP	Post-exposure prophylaxis
RIG	Rabies immune globulin
ERIG	Equine rabies immune globulin
HRIG	Human rabies immune globulin
GMC	Geometric mean concentration
CI	Confidence interval
RFFIT	Rapid fluorescence focusing inhibition test
RVNA	Rabies virus-neutralizing activity
IFA	Indirect immunofluorescence assay
ELISA	Enzyme-linked immunosorbent assay
scFv	Single-chain antibody fragment
CVS-11	Standard challenge rabies virus strain
PCR	Polymerase chain reaction
NCPC	North China Pharmaceutical Group Corporation
MTTI	Molecular Targeting Technologies
ChiCTR	Chinese Clinical Trial Registry
PK	Pharmacokinetics
PD	Pharmacodynamics

References

- Baltimore D. Expression of animal virus genomes. *Bacteriol Rev.* 1971;35(3):235–41. doi:10.1128/br.35.3.235-241.1971.
- Wang MH, Zhu HW, He MH, Wen YJ, Cheng SP. Advance in biological function of rabies virus glycoprotein. *China Anim Husb Vet Med.* 2016;43(12):3349–55. in Chinese.
- Lv XJ, Ma XJ, Wang LH, Li H, Shen XX, Yu PC, Tang Q, Liang GD. Preparation and initial application of a monoclonal antibody specific for a newly discovered conserved linear epitope of rabies virus nucleoprotein. *Biomed Environ Sci.* 2012;25(1):98–103. doi:10.3967/0895-3988.2012.01.014.
- Lafon M, Wiktor TJ, Macfarlan RI. Antigenic sites on the CVS rabies virus glycoprotein: analysis with monoclonal antibodies. *J Gen Virol.* 1983;64(Pt 4):843–51. doi:10.1099/0022-1317-64-4-843.

5. Dietzschold B, Gore M, Casali P, Ueki Y, Rupprecht CE, Notkins AL, Koprowski H. Biological characterization of human monoclonal antibodies to rabies virus. *J Virol.* 1990;64(6):3087–90. doi:10.1128/jvi.64.6.3087-3090.1990.
6. Lafon M, Edelman L, Bouvet JP, Lafage M, Montchâtre E. Human monoclonal antibodies specific for the rabies virus glycoprotein and N protein. *J Gen Virol.* 1990;71(Pt 8):1689–96. doi:10.1099/0022-1317-71-8-1689.
7. Yousaf MZ, Qasim M, Zia S, Khan M, Ashfaq UA, Khan S. Rabies molecular virology, diagnosis, prevention and treatment. *Viol J.* 2012;9:50. doi:10.1186/1743-422X-9-50.
8. Wilkerson JA. Rabies update. *Wilderness Environ Med.* 2000;11(1):31–39. doi:10.1580/1080-6032(2000)011[0031:RU]2.3.CO;2.
9. World Health Organization. Rabies post-exposure prophylaxis. 2014 [accessed 2021 July 21]. https://apps.who.int/iris/bitstream/handle/10665/85346/9789245209829_chi.pdf;sequence=9.
10. Both L, Banyard AC, van Dolleweerd C, Horton DL, Ma JK, Fooks AR. Passive immunity in the prevention of rabies. *Lancet Infect Dis.* 2012;12(5):397–407. doi:10.1016/S1473-3099(11)70340-1.
11. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, Barrat J, Blanton JD, Briggs DJ, Cleaveland S, et al. Estimating the global burden of endemic canine rabies. *PLoS Negl Trop Dis.* 2015;9(4):e0003709. doi:10.1371/journal.pntd.0003709.
12. Carrieri ML, Peixoto ZM, Paciencia ML, Kotait I, Germano PM. Laboratory diagnosis of equine rabies and its implications for human postexposure prophylaxis. *J Virol Methods.* 2006;138(1–2):1–9. doi:10.1016/j.jviromet.2006.07.005.
13. de Kruif J, Bakker AB, Marissen WE, Kramer RA, Throsby M, Rupprecht CE, Goudsmit J. A human monoclonal antibody cocktail as a novel component of rabies postexposure prophylaxis. *Annu Rev Med.* 2007;58:359–68. doi:10.1146/annurev.med.58.061705.145053.
14. Satpathy DM, Sahu T, Behera TR. Equine rabies immunoglobulin: a study on its clinical safety. *J Indian Med Assoc.* 2005;103:238, 241–232.
15. Goudsmit J, Marissen WE, Weldon WC, Niezgoda M, Hanlon CA, Rice AB, Kruif J, Dietzschold B, Bakker AB, Rupprecht CE. Comparison of an anti-rabies human monoclonal antibody combination with human polyclonal anti-rabies immune globulin. *J Infect Dis.* 2006;193(6):796–801. doi:10.1086/500470.
16. De Benedictis P, Minola A, Rota Nodari E, Aiello R, Zecchin B, Salomoni A, Foglierini M, Agatic G, Vanzetta F, Lavenir R, et al. Development of broad-spectrum human monoclonal antibodies for rabies post-exposure prophylaxis. *EMBO Mol Med.* 2016;8(4):407–21. doi:10.15252/emmm.201505986.
17. Gogtay N, Thatte U, Kshirsagar N, Leav B, Molrine D, Cheslock P, Kapre SV, Kulkarni PS. Safety and pharmacokinetics of a human monoclonal antibody to rabies virus: a randomized, dose-escalation phase I study in adults. *Vaccine.* 2012;30(50):7315–20. doi:10.1016/j.vaccine.2012.09.027.
18. Gogtay NJ, Munshi R, Ashwath Narayana DH, Mahendra BJ, Kshirsagar V, Gunale B, Moore S, Cheslock P, Thaker S, Deshpande S, et al. Comparison of a novel human rabies monoclonal antibody to human rabies immunoglobulin for postexposure prophylaxis: a phase 2/3, randomized, single-blind, noninferiority, controlled study. *Clin Infect Dis.* 2018;66(3):387–95. doi:10.1093/cid/cix791.
19. The Clinical Trials Registry India (CTRI). A randomized, double-blind, placebo-controlled phase I/II study to evaluate the safety, tolerability and neutralizing activity of Rabimabs (A murine anti-rabies monoclonal antibody cocktails) against rabies virus in healthy subjects. 2016 November 16 [accessed 2021 July 22]. <http://ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=11852&EncHid=&userName=Rabimabs>.
20. Kansagra K, Parmar D, Mendiratta SK, Patel J, Joshi S, Sharma N, Parihar A, Bhoge S, Patel H, Kalita P, et al. A phase 3, randomised, open-label, non-inferiority trial evaluating anti-rabies monoclonal antibody cocktail (Twinrab™) against Human Rabies Immunoglobulin (HRIG). *Clin Infect Dis.* 2021;73(9):e2722–e2728.
21. ClinicalTrials.gov. Safety and immunogenicity study of recombinant human rabies immunoglobulin (rhRIG) in combination with rabies vaccine for human use with human rabies immune globulin (HRIG) in combination with rabies vaccine for human use in healthy adult subjects. 2015 Sept 25 [accessed 2021 Sept 17]. <https://clinicaltrials.gov/ct2/show/study/NCT02559921>.
22. Chinese Clinical Trial Registry (ChiCTR). Preliminary assessment of the dose tolerance, safety, pharmacokinetics and neutralizing activity of rhRIG. 2019 Jun13 [accessed 2021 Sept 15] <http://www.chictr.org.cn/showproj.aspx?proj=39967>.
23. Chinese Clinical Trial Registry (ChiCTR). Assessment of the safety, pharmacokinetics, neutralizing activity and immunogenicity of rhRIG combined with rabies vaccine. 2019 Jun 11 [accessed 2021 Sept 15]. <http://www.chictr.org.cn/showproj.aspx?proj=36532>.
24. Chinese Clinical Trial Registry (ChiCTR). Evaluation of the efficacy and safety of rhRIG combined with rabies vaccine and the HRIG combined with rabies vaccine. 2019 May 17 [accessed 2021 Sept 15]. <http://www.chictr.org.cn/showproj.aspx?proj=36372>.
25. Chinese Clinical Trial Registry (ChiCTR). Evaluation of the efficacy and safety of rhRIG combined with rabies vaccine and the marketed HRIG combined with rabies vaccine: a randomized, blind, positive controlled trial. 2019 Feb 23 [accessed 2021 Sept 15]. <http://www.chictr.org.cn/showproj.aspx?proj=36013>.
26. ClinicalTrials.gov. A comparison of the safety, PD and PK of a single dose of SYN023 administered with licensed rabies vaccines. 2016 Nov 6. [accessed 2021 Sept 17]. <https://clinicaltrials.gov/ct2/show/study/NCT02956746>.
27. McClain JB, Chuang A, Moore SM, Tsao E. Safety, pharmacokinetics, and neutralizing activity of SYN023, a mixture of two novel antirabies monoclonal antibodies intended for use in postrabies exposure prophylaxis. *Clin Pharmacol Drug Dev.* 2021;10(7):807–17. doi:10.1002/cpdd.917.
28. ClinicalTrials.gov. A phase III clinical study to evaluate SYN023's efficacy and safety. 2020 Nov 25 [accessed 2021 Sept 17]. <https://clinicaltrials.gov/ct2/show/study/NCT04644484>.
29. Bakker AB, Python C, Kissling CJ, Pandya P, Marissen WE, Brink MF, Lagerwerf F, Worst S, van Corven E, Kostense S, et al. First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. *Vaccine.* 2008;26(47):5922–27. doi:10.1016/j.vaccine.2008.08.050.
30. ClinicalTrials.gov. Randomized phase II trial on safety and neutralizing activity of CL184 and rabies vaccine versus human rabies immune globulin (HRIG) and rabies vaccine in children and adolescents. 2008 Jul 2 [accessed 2021 Sept 18]. <https://clinicaltrials.gov/ct2/show/study/NCT00708084>.
31. ClinicalTrials.gov. Rabies virus neutralizing activity and safety of CL184, a monoclonal antibody cocktail, in simulated rabies post exposure prophylaxis in healthy adults. 2010 Oct 26 [accessed 2021 Sept 18]. <https://clinicaltrials.gov/ct2/show/study/NCT01228383>.
32. Rabishield rabies human monoclonal antibody. Serum Institute of India PVT.LTD. 2016 [accessed 2021 Jul 19]. https://www.seruminstitute.com/product_ind_rabishield.php.
33. Twinrab™ (RabiMabs) rabies human monoclonal antibody. Zyds Cadila in India. 2019 [accessed 2021 Jul 19]. <https://twinrab.com>.
34. Sloan SE, Hanlon C, Weldon W, Niezgoda M, Blanton J, Self J, Rowley KJ, Mandell RB, Babcock GJ, Thomas WDJ, et al. Identification and characterization of a human monoclonal antibody that potentially neutralizes a broad panel of rabies virus isolates. *Vaccine.* 2007;25(15):2800–10. doi:10.1016/j.vaccine.2006.12.031.
35. McMichael JC, Fiske MJ, Fredenburg RA, Chakravarti DN, VanDerMeid KR, Barniak V, Caplan J, Bortell E, Baker S, Arumugham R, et al. Isolation and characterization of two

- proteins from *Moraxella catarrhalis* that bear a common epitope. *Infect Immun.* 1998;66(9):4374–81. doi:10.1128/IAI.66.9.4374-4381.1998.
36. Anwith HS, Ravish HS, DHJJoCH Ashwathnarayana. Safety of new indigenous human Rabies Monoclonal Antibody (RMAb) for post exposure prophylaxis corresponding author citation article cycle. *Indian J. Community Health.* 2018;30(3):196–201.
 37. Kuzmina NA, Kuzmin IV, Ellison JA, Rupprecht CE. Conservation of binding epitopes for monoclonal antibodies on the rabies virus glycoprotein. *J Antivir Antiretrovir.* 2013;5:037–043. doi:10.4172/jaa.1000061.
 38. Presentation from Zydus Cadila at the FDA workshop on rabies monoclonal antibody products as a component of rabies post-exposure prophylaxis. 2018 Jul 17 [accessed 5 Sept 2021]. <https://www.fda.gov/downloads/Drugs/NewsEvents/UCM566898.pdf>.
 39. Wiktor TJ, Koprowski H. Monoclonal antibodies against rabies virus produced by somatic cell hybridization: detection of antigenic variants. *Proc Natl Acad Sci U S A.* 1978;75(8):3938–42. doi:10.1073/pnas.75.8.3938.
 40. Muhamuda K, Madhusudana SN, Ravi V. Use of neutralizing murine monoclonal antibodies to rabies glycoprotein in passive immunotherapy against rabies. *Hum Vaccin.* 2007;3(5):192–95. doi:10.4161/hv.3.5.4386.
 41. Cheng W, Yu H, Niu WB, Tang GQ. Preparation and identification of monoclonal antibodies against rabies virus glycoprotein. *Vet Orientation.* 2019;22:217–18. (in Chinese).
 42. Ueki Y, Goldfarb IS, Harindranath N, Gore M, Koprowski H, Notkins AL, Casali P. Clonal analysis of a human antibody response. Quantitation of precursors of antibody-producing cells and generation and characterization of monoclonal IgM, IgG, and IgA to rabies virus. *J Exp Med.* 1990;171(1):19–34. doi:10.1084/jem.171.1.19.
 43. Wang MX, Jia M, Jin M, Han J, Duan J, Wang LH, Jing RH, Li N, Yao JL, Li YF, et al. Safety of a single injection with recombinant human rabies immunoglobulin at various dosages in humans. *Chin J Biologicals.* 2013;26(7):95–99. (in Chinese).
 44. Marissen WE, Kramer RA, Rice A, Weldon WC, Niezgoda M, Faber M, Slootstra JW, Meloen RH, Clijsters-van der Horst M, Visser TJ, et al. Novel rabies virus-neutralizing epitope recognized by human monoclonal antibody: fine mapping and escape mutant analysis. *J Virol.* 2005;79(8):4672–78. doi:10.1128/JVI.79.8.4672-4678.2005.
 45. Bakker AB, Marissen WE, Kramer RA, Rice AB, Weldon WC, Niezgoda M, Hanlon CA, Thijsse S, Backus HH, de Kruif J, et al. Novel human monoclonal antibody combination effectively neutralizing natural rabies virus variants and individual in vitro escape mutants. *J Virol.* 2005;79(14):9062–68. doi:10.1128/JVI.79.14.9062-9068.2005.
 46. Cheng Z, Sun LN, Liang MF. Progress of therapeutic human anti-rabies antibodies. *Chin Med Biotechnol.* 2010;5(3):208–10. (in Chinese).
 47. Vaughan TJ, Osbourn JK, Tempest PR. Human antibodies by design. *Nat Biotechnol.* 1998;16(6):535–39. doi:10.1038/nbt0698-535.
 48. Kramer RA, Marissen WE, Goudsmit J, Visser TJ, Clijsters-van der Horst M, Bakker AQ, de Jong M, de Jongeneelen M, Thijsse S, Backus HH, et al. The human antibody repertoire specific for rabies virus glycoprotein as selected from immune libraries. *Eur J Immunol.* 2005;35(7):2131–45. doi:10.1002/eji.200526134.
 49. Zhao XL, Yin J, Wang H, Jiang M, Hou XJ. Construction of human phage-displayed scFv library and selection of the ScFv against rabies virus. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.* 2004;20:243–47.
 50. Zhao XL, Yin J, Chen WQ, Jiang M, Yang G, Yang ZH. Generation and characterization of human monoclonal antibodies to G5, a linear neutralization epitope on glycoprotein of rabies virus, by phage display technology. *Microbiol Immunol.* 2008;52(2):89–93. doi:10.1111/j.1348-0421.2008.00016.x.
 51. YU L, Sun L, Jin J, Li C, Li DX, Liang MF. Generation of recombinant human ScFv antibodies against Rabies virus. *Chinese J Exp Clin Virol.* 2012;26(3):189–92. (in Chinese).
 52. Fang SD, Zhao XR, Chen JJ, An C, Nan JJ, Mao XY. Construction and validation of humanized anti-rabies virus monoclonal antibodies. *Prog Microbiol Immunol.* 2019;47(4):7–13. (in Chinese).
 53. Houmel M, Dellagi K. Isolation and characterization of human neutralizing antibodies to rabies virus derived from a recombinant immune antibody library. *J Virol Methods.* 2009;161(2):205–15. doi:10.1016/j.jviromet.2009.06.018.
 54. Sun L, Chen Z, Yu L, Wei J, Li C, Jin J, Shen X, Lv X, Tang Q, Li D, et al. Generation and characterization of neutralizing human recombinant antibodies against antigenic site II of rabies virus glycoprotein. *Appl Microbiol Biotechnol.* 2012;96(2):357–66. doi:10.1007/s00253-012-4171-4.
 55. Zhao XL, Chen WQ, H F, Shen CF, Ji Y, Li JM, Zhang SJ, Yang ZH. Preparation of human antibody fragments against rabies virus based on ribosome display technology. *Prog Biochem Biophys.* 2011;38(10):945–52. (in Chinese). doi:10.3724/SP.J.1206.2010.00521.
 56. Groves M, Lane S, Douthwaite J, Lowne D, Rees DG, Edwards B, Jackson RH. Affinity maturation of phage display antibody populations using ribosome display. *J Immunol Methods.* 2006;313(1–2):129–39. doi:10.1016/j.jim.2006.04.002.
 57. Zhao XL, Chen WQ, Yang ZH, Li JM, Zhang SJ, Tian LF. Selection and affinity maturation of human antibodies against rabies virus from a scFv gene library using ribosome display. *J Biotechnol.* 2009;144(4):253–58. doi:10.1016/j.jbiotec.2009.09.022.
 58. Hellert J, Buchrieser J, Larrous F, Minola A, de Melo GD, Soriaga L, England P, Haouz A, Telenti A, Schwartz O, et al. Structure of the prefusion-locking broadly neutralizing antibody RVC20 bound to the rabies virus glycoprotein. *Nat Commun.* 2020;11(1):596. doi:10.1038/s41467-020-14398-7.
 59. Molecular Targeting Technologies. Rabies mAb product. [accessed 2021 Sept 23]. <http://www.mtarget.com/mm5/pdfs/pipeline/RabiesMonoclonalAnti.pdf>.
 60. Chao TY, Ren S, Shen E, Moore S, Zhang SF, Chen L, Rupprecht CE, Tsao E. SYN023, a novel humanized monoclonal antibody cocktail, for post-exposure prophylaxis of rabies. *PLoS Negl Trop Dis.* 2017;11(12):e0006133. doi:10.1371/journal.pntd.0006133.
 61. Chao TY, Zhang SF, Chen L, Tsao E, Rupprecht CE. In vivo efficacy of SYN023, an anti-rabies monoclonal antibody cocktail, in post-exposure prophylaxis animal models. *Trop Med Infect Dis.* 2020;5:31–39.
 62. Ding Y, Wu M, Zhang H, Zhu X, Hu Y, Li X, Liu J, Tsao E, Liu M, Li C. Safety, pharmacokinetics and pharmacodynamics of SYN023 alone or in combination with a rabies vaccine: an open, parallel, single dose, phase I bridging study in healthy Chinese subjects. *Antiviral Res.* 2020;184:104956. doi:10.1016/j.antiviral.2020.104956.
 63. Franka R, Carson WC, Ellison JA, Taylor ST, Smith TG, Kuzmina NA, Kuzmin IV, Marissen WE, Rupprecht CE. In vivo efficacy of a cocktail of human monoclonal antibodies (CL184) against diverse North American bat rabies virus variants. *Trop Med Infect Dis.* 2017;2:48–59.
 64. Sparrow E, Torvaldsen S, Newall AT, Wood JG, Sheikh M, Kieny MP, Abela-Ridder B. Recent advances in the development of monoclonal antibodies for rabies post exposure prophylaxis: a review of the current status of the clinical development pipeline. *Vaccine.* 2019;37(Suppl 1):A132–a139. doi:10.1016/j.vaccine.2018.11.004.
 65. Sun LN, Liu Y, Li C, Li DX, Liang MF. Generation of human scfv antibodies for antigenic site III of rabies virus glycoprotein from antibody-phage libraries by chain shuffling. *Chin J Virol.* 2016;32(4):393–98. (in Chinese).
 66. Sun LN, Liu Y, Li C, Li DX, Liang MF. Development of recombinant human monoclonal antibody cocktail for post-exposure rabies prophylaxis. *Chin J Virol.* 2016;32(4):399–403. (in Chinese).