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Monoclonal antibodies against rabies: current uses in prophylaxis and in therapy

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Rabies is a severe viral infection that causes an acute encephalomyelitis, which presents a case fatality of nearly 100% after the manifestation of neurological clinical signs. Rabies can be efficiently prevented with post-exposure prophylaxis (PEP), composed of vaccines and anti-rabies immunoglobulins (RIGs); however, no treatment exists for symptomatic rabies. The PEP protocol faces access and implementation obstacles in resource-limited settings, which could be partially overcome by substituting RIGs for monoclonal antibodies (mAbs). mAbs offer lower production costs, consistent supply availability, long-term storage/stability, and an improved safety profile. Here we summarize the key features of the different available mAbs against rabies, focusing on their application in PEP and highlighting their potential in a novel therapeutic approach.

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Introduction

Rabies is a fatal, acute and progressive encephalomyelitis caused by neurotropic viruses from the *Rhabdoviridae* family belonging to the *Lyssavirus* genus (from the Greek lyssa: fury or madness). This disease is an anthropozoonosis that affects a wide range of carnivores, which act as primary hosts of lyssaviruses, namely dogs, foxes, raccoons, skunks, mongooses and diverse species of bats [1]. Rabies is present worldwide and it is estimated to cause about 60 000 human deaths each year. It is considered one of the most neglected diseases, with no treatment existing for those patients who manifest neurological clinical signs [2,3].

Rabies virus (RABV) is the type species of this genus, which currently contains a total of 17 recognized species, distributed in two major phylogroups according to their viral genetic distances: phylogroup 1 (Aravan lyssavirus, Australian bat lyssavirus, Bokeloh bat lyssavirus, Duvenhage lyssavirus, European bat lyssavirus 1, European bat lyssavirus 2, Gannoruwa bat lyssavirus, Irkut lyssavirus, Khujand lyssavirus, Rabies lyssavirus, and Taiwan bat lyssavirus) and phylogroup II (Lagos bat lyssavirus, Mokola lyssavirus, and Shimoni bat lyssavirus). Three additional species (Ikoma lyssavirus, Lleida bat lyssavirus, and West Caucasian bat virus) are the most genetically divergent lyssaviruses and do not fall into either of the two phylogroups [4–8].

The transmission of RABV to humans occurs by crossing the cutaneous barrier, most commonly via the saliva of an infected animal, but transmission via direct projection over mucous membranes (eyes, nose, mouth) or organ transplantation from infected donors may also occur [3,9]. RABV will initially infect and replicate in myocytes at the site of infection and will then infect motor neurons, passing through the motor endplates mainly via nicotinic acetylcholine receptors (nAChRs) [10]. Other molecules such as the neural cell adhesion molecule (NCAM or CD56), the low-affinity nerve-growth factor receptor (p75NTR), the metabotropic glutamate receptor subtype 2 (mGluR2), integrin β1 (ITGB1) and cellular heparan sulfate (HS) may act as receptors or attachment factors for RABV [11–15]. Rabies virus will then travel retrogradely via the axons to the spinal cord and further up to the brain [9,16]. The incubation period of rabies is rather variable, ranging from a few days up to several years, nevertheless, the average is two to three months. The time at which clinical signs are manifested can be correlated to RABV dissemination throughout the central nervous system [9,17]. In spite of new epidemiological cycles emerging regularly due to the evolutionary dynamics observed in lyssaviruses and regardless of their capacity to cross species barriers and infect many different species of mammals [18], RABV transmitted by domestic dogs is

responsible for more than 98% of human rabies deaths around the world [2,3].

Lyssaviruses are enveloped and negative-sensed, single-stranded RNA viruses. The genome is approximately 12 kb large and encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the RNA polymerase (L) [1]. The N protein encapsidates the viral genome, constituting the ribonucleoprotein, which in turn interacts with the P and L proteins to form the viral nucleocapsid. The M protein binds to the inner face of the envelope, making a connection between the envelope and the ribonucleoprotein complex. Finally, the G protein is arranged in trimeric spikes on the surface of the viral envelope, where it is targeted by neutralizing antibodies [19] (Figure 1).

This review will present the current status and recent advances in the development of monoclonal antibodies (mAbs) in rabies prophylaxis and therapy with a focus on those that have demonstrated efficacy in preclinical animal models or in clinical trials. The review is divided into five chapters: the structure of the RABV antigenic sites, a reminder of the current rabies control and prevention measures, the use of mAbs in rabies prophylaxis, the use of mAbs in rabies therapy and the perspectives of using mAbs in rabies control.

Structure of the rabies virus glycoprotein, the target of neutralizing antibodies

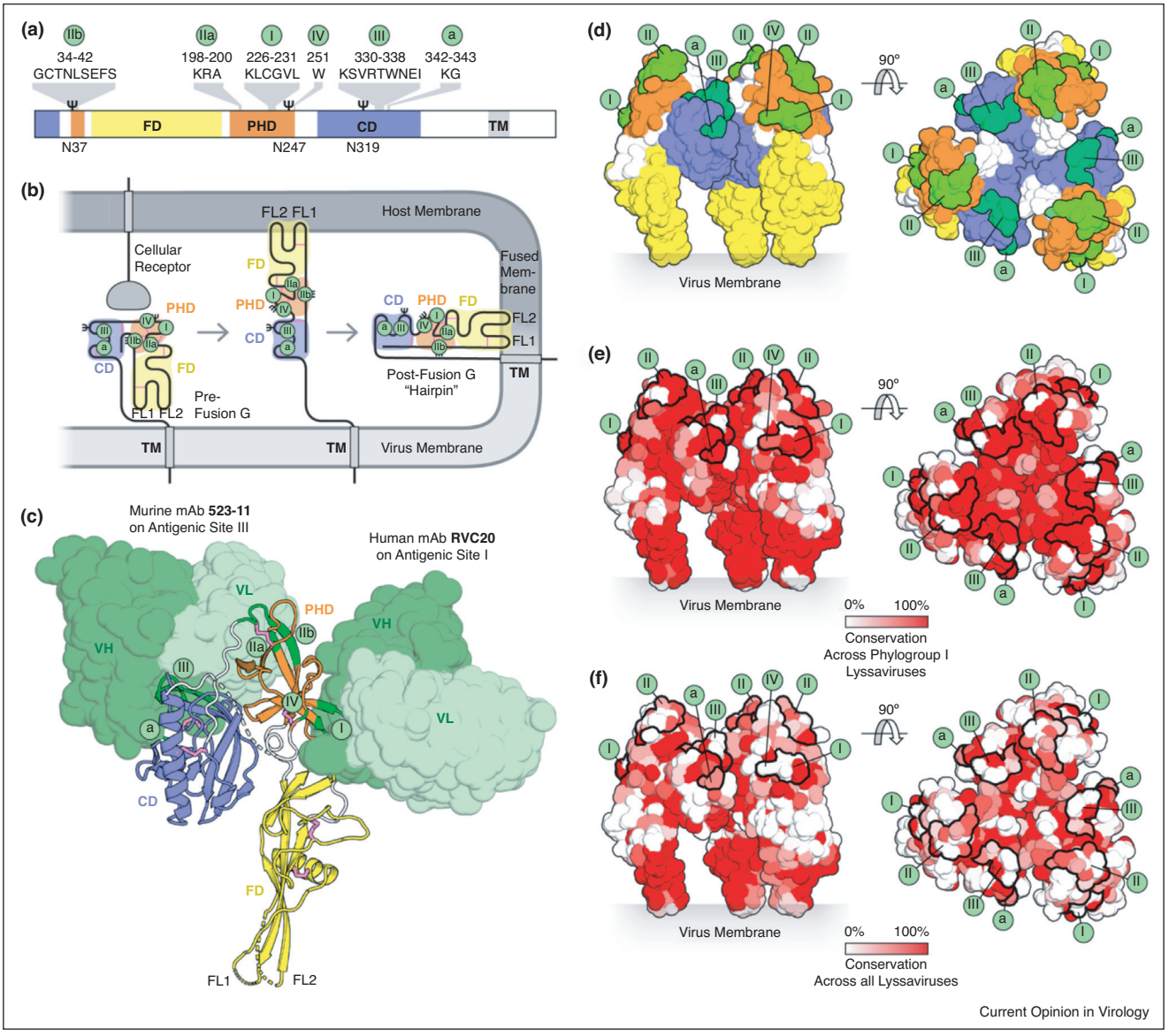
Rabies virus G is the only viral antigen exposed on the surface of virus particles and is the principal target of the host's neutralizing humoral immune response. G is 505 amino acid residues long and contains a single trans-membrane helix near its carboxy-terminus that anchors it to the viral envelope (Figure 1a). The amino-terminal 440 residues preceding this trans-membrane helix form its ectodomain, which protrudes from the viral envelope and is directly accessible to antibodies. The ectodomain typically carries three asparagine-linked glycans at positions 37, 247 and 319, and it is stabilized by seven disulfide bonds.

G protein has functions in receptor binding and in membrane fusion, both of which may be inhibited by antibody binding. It belongs to the class III of viral membrane fusion proteins [20••] and as such, shares a common domain topology with the fusion proteins of other rhabdoviruses, such as vesicular stomatitis virus (VSV) [21] and Chandipura virus [22], but also with baculoviruses [23] and herpesviruses [24]. The G ectodomain can be further subdivided into three subdomains called the fusion domain (FD), the Pleckstrin homology domain (PHD) and the central domain (CD), which are connected to each other via flexible hinges (Figure 1a–d). During cell entry, endosomal acidification triggers a series of conformational changes in these hinges, which at first lead to the exposure of two fusion loops at the extremity of the fusion domain and their insertion into a host membrane. The protein next forms a hairpin that brings the host membrane-anchored fusion loops into close proximity of the envelope-anchored trans-membrane helix (Figure 1b). This structural rearrangement ultimately leads to membrane fusion and to the release of the virus' genetic material into the cytoplasm [20••]. Although the currently available crystal structures of the Rabies G ectodomain show the protein only in a monomeric state (Figure 1c), electron microscopy of whole virions has provided evidence that full-length native G acts as a trimer [25] (Figure 1d).

Potently neutralizing antibodies target five antigenic sites that are distributed across the membrane-distal half of the G structure (Figure 1b–f). These sites are denoted I, IIa/IIb, III, IV and 'a' [26]. Neutralizing antibodies from human vaccinees primarily react with sites I and III [27] and it is reasonable to assume that these are also the main antigenic sites upon infection. Our structural and functional analysis of the site I-binding broadly neutralizing human mAb RVC20 has demonstrated that it locks the hinge between subdomains PHD and FD, thus inhibiting a critical conformational movement during membrane fusion [28••] (Figure 1c). This neutralization mechanism is likely also shared with other site I antibodies. In addition, the structure of the site III-binding murine mAb 523-11 has been determined in complex with G [20••] (Figure 1c), likely employing a different neutralization mechanism: although the quaternary structure of the native Rabies G trimer is not yet available, comparison to the homologous trimeric crystal structure of VSV G suggests that mAb 523-11 binding disrupts the G trimer by sterical displacement of a neighboring protomer. Such trimerization inhibition is another neutralization mechanism targeting the fusion machinery that is likely shared among many site III antibodies. The extent to which the various neutralizing antibodies also inhibit receptor binding still needs to be investigated.

The emergence of non-Rabies lyssaviruses with pathogenicity in humans has motivated efforts to select mAbs that do not only neutralize RABV but also its phylogenetic relatives. The most broadly neutralizing antibodies identified thus far show robust reactivity with most phylogroup I viruses [27,29,30]. However, although a pan-Lyssavirus mAb with neutralizing activity against all Lyssaviruses would be desirable, such antibodies will be exceedingly challenging to find: whereas the antigenic surface of the membrane-distal part of the G spike is reasonably well conserved across phylogroup I lyssaviruses, it is highly divergent across the entire genus (Figure 1e–f). On the contrary, antibodies targeting the most conserved regions in the membrane-proximal region of the spike can be expected to have a strong preference for low-pH forms of G, in which these parts are more

Figure 1



Architecture and antigenicity of Rabies virus G.
(a) Positions and sequences of the antigenic sites. *N*-glycosylation sites are indicated by Ψ. Numbering corresponds to the mature protein after cleavage from its secretion signal peptide. **(b)** Simplified schematic of the acid-triggered membrane fusion mechanism of G. Although only one protomer is shown, the protein acts as a trimer. **(c)** Pre-fusion structure of the G ectodomain in complex with the variable domains (VH and VL) of murine mAb 523-11 bound to antigenic site III and human mAb RVC20 bound to antigenic site I. The image was compiled from the coordinate files of PDB entries 6LGW, 6LGX and 6TOU [28••,86]. Disulfide bonds are drawn in magenta. **(d)** Model of the trimeric assembly of the Rabies virus G ectodomain based on homology to the trimeric crystal structure of the VSV G ectodomain (PDB: 5I2S) [21] **(e)** Surface conservation across phylogroup I lyssaviruses. **(f)** Surface conservation across all lyssaviruses. Antigenic sites are outlined in black. Color plots were generated with ESPript 3 [87].

exposed, and will therefore be expected to display a low neutralizing activity.

Current rabies control and prevention measures

Rabies is a 100% vaccine-preventable disease. To control human rabies, vaccination of dogs' population is one of the most effective measures. After exposure to RABV, the disease is still fully preventable in humans by early post-exposure management, which comprises washing and disinfection of wounds and post-exposure prophylaxis (PEP), which includes vaccines and anti-rabies immunoglobulins (RIGs) (Table 1). The clinical and epidemiological evaluation of the categories of contact and the PEP

decision is made in specialized anti-rabies centers or integrated bite management centers [3].

RIGs are delivered in most severely exposed patients (Table 1). The interest in using these immunoglobulins as a complementary measure to the original vaccine was demonstrated by several studies before the 1970's [31–38], and their use was first recommended by the World Health Organization (WHO) in 1975 [39]. The administration of RIGs around the wound is preferable to be performed at the beginning of the PEP protocol, otherwise as soon as possible after the initiation of PEP, but never later that 7 days after the initiation of the PEP, due to possible interference with vaccine. The use of RIGs provides an immediate effect by neutralizing the infectivity of virions at the site of infection during the first days post-exposure, at a time when the adaptive immune response induced by the vaccine injection is still not effective [3], however, the use of RIGs is not recommended in patients who received a previous pre-exposure prophylaxis or PEP.

The first RIG product available was composed of equine rabies immunoglobulins (ERIGs), obtained from hyperimmunized horses. Presently, ERIGs, human rabies immunoglobins (HRIGs), and highly purified $F(ab')_2$ fragments from ERIGs [40] are available on the market. The maximum dose of HRIG is 20 IU/kg of body weight, while that of ERIG and $F(ab')_2$ fragments is 40 IU/kg of body weight. However, there are several obstacles in the widespread implementation and uptake of RIGs, including cost, supply shortages (particularly in rural areas), and potential safety issues with blood derived products. As a result, in Africa, RIG is used in less than 10% of the exposures that would require their administration according to WHO recommendations [41••].

Monoclonal antibodies in rabies prophylaxis

The development of monoclonal antibodies (mAbs) against rabies has been fomented since the 1990's by the WHO, in order to have an alternative to the use of RIGs in the PEP protocol [42]. mAbs potential advantages over HRIG and ERIG are : greater breadth, lower dose, improved safety, longer shelf life, ease of administration, lower cost of production, ability for large scale production, and consistency in product. Further, RABV neutralizing mAbs can be obtained from the humanization of previously identified mouse mAbs, developed as fully human mAbs derived from vaccinated individuals or produced as fully human mAbs in transgenic mice expressing human antibody gene sequences [43]. However, each mAb presents a mono-specificity, and therefore it is recommended that cocktails containing at least two mAbs binding to non-overlapping epitopes should be used, to limit the risk of failure due to lack of coverage of circulating RABV strains or to emergent viral escapes [3,44,45].

Despite the medical urgency of providing quality biologics for rabies control and the growing market for mAbs in human medicine, including in the area of infectious diseases [46,47,48••], only two PEP products containing mAbs are available to fight rabies, both of them licensed only in the Indian market (Table 2).

The first mAb is Rabishield, a product containing one single recombinant human mAb (IgG1) against a conformational epitope of the RABV G protein (which was first called 17C7, and later renamed as SII RMab or RAB1) [49], which was approved to be used in humans in 2016 [50]. Rabishield has been described to present a wide spectrum of neutralization over RABV isolates of public health importance from Americas, Europe, Africa and Asia [51]. However, due to its mono-specificity, a major concern of this product is the potential lack of neutralization of emerging rabies variants as well as the risk for selection of viral escape mutants [52•]. Indeed, the Rabishield mAb poorly neutralizes RABVs carrying the N336D mutation in G protein, which is detected in about 60% of the African isolates, and which has been increasingly detected in North America [27,53,54].

The second available mAb product against rabies is Twinrab, licensed in 2019 [55], and which contains two recombinant mouse mAb: M777-16-3 or Miromavimab, an IgG1 that binds to the antigenic site II of the RABV G protein, and 62-71-3 or Docaravimab, an IgG2b that binds to the antigenic site III of the rabies virus glycoprotein

Table 1

Categories of contact and recommended post-exposure prophylaxis (PEP)

Categories of contact with suspect rabid animal	Post-exposure prophylaxis measures
Category I – touching or feeding animals, animal licks on intact skin (no exposure)	Washing of exposed skin surfaces, no PEP
Category II – nibbling of uncovered skin, minor scratches or abrasions without bleeding (exposure)	Wound washing and immediate PEP with vaccine
Category III – single or multiple transdermal bites or scratches, contamination of mucous membrane or broken skin with saliva from animal licks, exposures due to direct contact with bats (severe exposure)	Wound washing, immediate PEP with vaccine and administration of rabies immunoglobulin

Source: WHO [3].

Table 2

Monoclonal antibodies against Rabies tested in preclinical studies or under clinical trials

mAbs identification	Antigenic site targets on RABV glycoprotein	Development stage	References
Rabishield (single human mAb, first called 17C7, then SII RMab)	Antigenic site III	Available in the Indian market since 2017	[49,50]
Twinrab (two mice mAbs M777-16-3 and 62-71-3, first called Rabimabs)	Antigenic sites II and III	Available in the Indian market since 2020	[55,57]
SYN023 (two humanized mAbs CTB011 and CTB012)	Antigenic sites III and IV	Phases I and II completed, Phase III ongoing	[58,61,62]
CL184 (two human mAbs CR57 and CR4098)	Antigenic sites I and III	Phases I and II completed, withdrawn	[66]
RVC20 and RVC58 (two human mAbs)	Antigenic sites I and III	Preclinical	[27,28••]
11B6 and NP-19-9 (two human mAbs)	Antigenic sites II and III	Preclinical	[30]
CR57, RV08 and RV3A5 (three human mAbs)	Antigenic sites I, II and III	Preclinical, more communications needed	[70]
Rabies mAb	*Information not publicly available*	Uncertain, probably in Phase III	[52•]

[56]. Both products, Rabishield and Twinrabs, presented comparable results in comparison with RIGs when used in PEP [49,57]. However, the precise dosage of mAbs required for passive immunization in the context of PEP still needs some specific studies [3].

Currently, there are other mAbs cocktails under development, with promising results in preclinical models or already being tested in human clinical trials (Table 2). One of these products is SYN023, composed of two humanized mAbs, CTB011 and CTB012, (IgG1), derived from the murine mAbs 3D11E3 and 7G11A [29], which recognize non-overlapping epitopes on the RABV G protein. Whereas CTB011 binds to the antigenic site III, the binding site of CTB012 is unknown. These mAbs were shown to neutralize Chinese and North American rabies virus isolates and to protect Syrian hamsters and dogs when used as PEP, however, CTB011 was not able to neutralize 3 out of 10 North American strains tested [58]. Phases I and II are completed for SYN023 [59,60•,61], and it is currently in a phase III clinical study [62].

Another mAbs cocktail composed of two human mAbs is CL184. CL184 contains CR57, an IgG1 binding to the antigenic site I of RABV G protein [63] and CR4098, a complementary IgG1 that binds to the antigenic site III [64]. Despite its broad neutralization activity, including over different bat RABV variants [65], and promising results in clinical trials [66,67], the development of this product was stopped [52•]. Another study using CR57, but in combination with two other recombinant human mAbs, RV08 (binds to the antigenic site II of R glycoprotein) [68] and RV3A5 (binds to the antigenic site III of rabies virus glycoprotein) [69], described that this triple cocktail was able to neutralize 11 RABV strains (vaccine, fixed and street strains) and to promote 100% survival of Syrian hamsters challenged with a lethal RABV dose [70]. However, there is a limited set of information concerning these mAbs available, and further studies are highly warranted [69,70].

RVC20 and RVC58 are two human mAbs (IgG1) that bind to antigenic sites I and III of the RABV G protein, respectively. Together, these mAbs were able to neutralize multiple RABV lineages and non-rabies virus lyssavirus species, and to protect Syrian hamsters from a lethal RABV challenge [27]. Similarly, the human mAbs 11B6 and NP-19-9 (IgG1) were recently described to potently neutralize a wide range of lyssavirus types (binding to the antigenic sites II and III of the RABV G protein, respectively) and to protect mice from lethal Indian RABV isolates (SV1-SV6) challenge [30].

Lastly, in 2016, the North China Pharmaceutical Group Corporation announced the phase I a completion of a Rabies mAb clinical trial, which is anti-rabies mAbs cocktail composed of two monoclonal antibodies [71]. This product seems to be currently in Phase III, as attested by the Molecular Targeting Technologies website [72], but more details in scientific publications are currently lacking [52•].

Monoclonal antibodies in rabies therapy

Despite being a fully preventable disease if early prophylactic measures are deployed, rabies is fatal in more than 99% of cases after the onset of clinical signs [9,17]. The current therapeutic approach, the Milwaukee protocol, was first described in 2004 as a possible method to manage symptomatic rabies. After some modifications, the current version of the Milwaukee protocol includes the induction of an artificial coma and the injection of ketamine and amantadine (two competitive antagonists of NMDA receptors that would prevent the release of viral particles) [73,74]. The efficacy of this protocol is uncertain with mixed results [75] and therefore the quest of an effective therapy remains an urgent medical need.

Following the promising effects of mAbs in rabies prophylaxis, the development of an anti-rabies immunotherapy has been described in a preclinical study using mice [76••]. This innovative protocol was based on a cocktail of two broadly neutralizing monoclonal antibodies, RVC20 and RVC58, whose positive activity has already being

described in the context of rabies post-exposure prophylaxis [27,28••] (Table 2).

The efficacy of this approach was based on a prolonged and continuous brain infusion (via the intracerebroventricular route) of the RVC20 and RVC58 mAbs cocktail combined with peripheral injections. This approach was efficient in curing rabid mice even at late stages of infection, when several animals already presented neurological signs. Part of the success of this treatment may be due to the need of central nervous system (CNS) penetration of the mAbs Fcγ receptor associate functions, resulting in a direct reduction of the CNS viral load during the symptomatic stage, combined with the ability of the mAbs to potentially modulate CNS inflammation mechanisms. Of note, peripheral administration only, or the use of Fc abrogated versions of the mAb cocktail did not yield therapeutic benefit [76••]. This is the first *in vivo* proof of concept for a treatment of symptomatic rabies with mAbs, which could be the premise of a new paradigm shift in the treatment of rabies in humans.

Perspectives

There are many reasons why replacing HRIG and ERIG with mAbs is not yet a reality on a global scale. Rabies is a disease present particularly in countries with low economic resources, and the phases of clinical trials for these categories of deadly diseases are complicated and require an important number of patients with a category III rabies exposure (n = 200 for Rabishield, n = 308 for Twinrab, and n = 1000 for SYN023). Regardless, in view of the many advantages inherent in the use of mAbs in PEP, the WHO does encourage the use of mAbs as an alternative to HRIG and ERIG [42].

In the context of rabies therapy, mAbs are promising compounds. Several therapeutic mAbs for other diseases are already in the market, especially for the treatment of cancer [77]. In infectious diseases, mAbs have also shown their utility including in the treatment of Ebola and COVID-19 [78,79]. Currently, mAbs production costs have drastically decreased, while production scale has increased [80], and mAbs technology and product development strategies have evolved to allow accelerated development, as demonstrated with COVID-19 [81]. Now, the use of mAbs to fight infectious diseases is a reality, with different identified products against infectious and neglected diseases such as dengue, malaria and schistosomiasis [82]. Complementary to chemical molecules, mAbs present favorable pharmacokinetics and pharmacodynamics properties (longer half-lives), reduced induction of side effects and remarkable specificity and affinity to the desired biological targets [83].

Efficient anti-rabies mAbs cocktails need to contain mAbs that potently and broadly neutralize rabies virus and other non-rabies virus lyssavirus species. With the advances in mAbs engineering, production of humanized and chimeric mAbs are now easy, and the generation of different forms of antibodies fragments, aiming to ameliorate mAbs' pharmacokinetics, to target epitopes more specifically, to explore effector functions is henceforth possible [76••,83–85], which open new avenues to the development of safe, efficient and cheaper mAbs to be used to fight rabies.

Conflict of interest statement

G.D.M., D.C and H.B. hold a patent for some of the mAbs described in the present review (PCT/EP2019/078439). D.C. and R.G are employees of Vir Biotechnology Inc. and may hold shares in Vir Biotechnology Inc.

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- • of special interest
- •• of outstanding interest

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