

# Monoclonal antibodies for the prevention of rabies: theory and clinical practice

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**Abstract:** Monoclonal antibodies (MAbs) have become a unique and attractive class of biologics, possessing several desirable characteristics for use in human medicine. Anti-infective MAbs for several medically important viral agents, including rabies virus (RABV), have been developed and are currently at different stages of clinical development. Rabies is a vaccine-preventable but neglected zoonosis. After severe bite exposures, prompt administration of a combination of potent rabies vaccine and rabies immunoglobulin (RIG) is recommended. Due in part to cost, equine RIG has been largely used instead of human RIG, especially in the developing world. With an estimated 10 million RABV exposures annually, the use of MAbs has emerged in concept as a potential alternative to polyclonal RIG for future prophylaxis needs. Murine MAbs, although efficacious, are less attractive because of immunogenicity. However, human MAbs seem to have the potential to replace polyclonal RIG because they possess all the desirable characteristics for an intended biologic. The exquisite specificity of the MAbs for a single epitope is generally believed to result in narrow spectrum of RABV neutralization and perceived generation of escape mutants. These issues can be mitigated by formulating a cocktail of candidate MAbs that are directed against distinct, nonoverlapping epitopes. Expression of recombinant human MAbs in mammalian cell lines, such as Chinese hamster ovary and human retinal, is central to the economical production at an industrial scale. Thus far, human MAbs developed by two companies have successfully passed through Phase I or II clinical trials in countries such as the US and India.

**Keywords:** rabies, postexposure prophylaxis, polyclonal RIG, monoclonal antibody, rabies immunoglobulin, clinical trials

## Introduction

Monoclonal antibodies (MAbs) are versatile molecules with an undisputed usefulness for biomedical applications. Besides their utility in diagnostic applications, MAbs are also attractive as therapeutic candidates because of their stability, tolerance, functionality, and amenability for engineering to enhance various desirable characteristics, such as reduced immunogenicity, longer half-lives, higher affinity, and better effector functions. Over the past decade, MAbs have become a thriving class of biologically active molecules for therapeutics.<sup>1</sup> Nearly one in five biotherapeutic molecules belongs to this category. Thus far, more than 25 antibodies have been licensed for human use (Table 1).<sup>2–12</sup> Nearly 200 other candidates are in different stages of development (Table 2). Though MAbs are indicated mainly for noninfectious diseases such as cancer, inflammatory conditions, and autoimmune disorders, they have also been investigated for their potential as anti-infective agents, although with limited success

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**Table 1** Selected examples of licensed therapeutic monoclonal antibodies

Name	Target	Year	Use	Manufacturer	Reference
Muronomab-CD3 (Orthoclone® OKT3)	CD3	1992	Transplant rejection	Centocor Ortho Biotech, Inc., Horsham, PA, USA	<sup>2</sup>
Rituximab (Rituxan)	CD20	1997	B cell non-Hodgkin lymphoma	Genentech Inc., San Francisco, CA, USA	<sup>3</sup>
Infliximab (Remicade)	TNF	1998	RA, ankylosing spondylitis, Crohn's disease, ulcerative colitis	Janssen Biotech, Inc., Horsham, PA, USA	<sup>4</sup>
Trastuzumab (Herceptin)	HER-2	1998	HER-2 positive breast cancer	Genentech Inc., San Francisco, CA, USA	<sup>5</sup>
Palivizumab (Synagis®)	RSV F protein	1998	Prevention of RSV infection in neonates	MedImmune, Gaithersburg, MD, USA	<sup>6</sup>
Alemtuzumab (Campath)	CD52	2001	B-CLL	Genzyme, Cambridge, MA, USA	<sup>7</sup>
Ibritumomab tiuxetan (Zevalin)	CD23	2002	Non-Hodgkin's lymphoma	Spectrum Pharmaceuticals, Irvine, CA, USA	<sup>8</sup>
Bevacizumab (Avastin)	VEGF	2004	Colorectal, lung, breast cancer	Genentech, San Francisco, SFO, USA	<sup>9</sup>
Panitumumab (Vectibix)	EGFR	2007	Colorectal cancer	Amgen Inc., Thousand Oaks, CA, USA	<sup>10</sup>
Ustekinumab (Stelara)	IL-12	2009	Plaque psoriasis	Janssen Biotech, Inc., Horsham, PA, USA	<sup>11</sup>
Mogamulizumab	CCR4	2012	Relapsed or refractory CCR4- positive T cell leukemia-lymphoma	Kyowa Hakko Kirin Co., Tokyo, Japan	<sup>12</sup>

**Abbreviations:** B-CLL, B-cell chronic lymphocytic leukemia; EGFR, epidermal growth factor receptor; IL, interleukin; RA, rheumatoid arthritis; RSV-F, respiratory syncytial virus fusion; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; CCR4, chemokine receptor 4; OKT, ortho kung t3; CD, cluster of differentiation; HER-2, human epidermal growth factor receptor 2.

to date. For example, of 46 anti-infective MAb tested clinically, only one, palivizumab, was approved by the US Food and Drug Administration as a prophylaxis for respiratory syncytial virus infection in high-risk pediatric patients. Of course, ample medical needs exist for the development of safe and efficacious targeted anti-infective MAb that would complement the current arsenal of vaccines and anti-infective

drugs.<sup>1</sup> Moreover, there is a renewed interest in the development of antiviral MAb because of potential safety issues and supply limitations associated with the use of polyclonal antibodies derived from human plasma. For the purpose of this review, our discussion is concentrated mainly upon the topic of antiviral MAb intended for the prevention of rabies in humans.

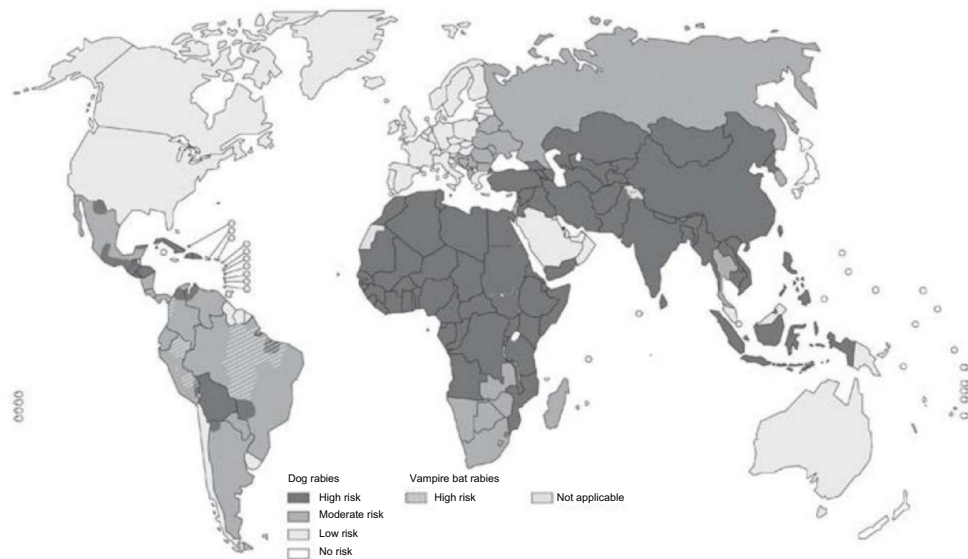
**Table 2** Selected examples of therapeutic monoclonal antibodies in clinical development

Name	Target	Phase	Use
CR6261/CR8020	Influenza HA type A	I	Influenza infection
Brentuximab vedotin	CD30	II	Germ cell cancer
Daratumumab	CD38	II	Multiple myeloma
Epratuzumab	CD22	II	B-cell acute lymphoblastic leukemia
MOR103	GM-CSF	II	Rheumatoid arthritis
Roledumab	Rhesus D	II	Prevention of Rhesus D alloimmunization
Evolocumab	PCSK-9	III	Hypercholesterolemia; hyperlipidemia
Gantenerumab	Amyloid-β	III	Alzheimer's disease
Ixekizumab	IL-17a	III	Psoriasis, psoriatic arthritis, plaque psoriasis
Tremelimumab	CTLA-4	III	Metastatic melanoma

**Abbreviations:** CTLA, cytotoxic T-lymphocyte antigen; GM-CSF, granulocyte-macrophage colony-stimulating factor; HA, hemagglutinin; IL, interleukin; PCSK, proprotein convertase subtilisin kexin; CD, cluster of differentiation.

## Postexposure prophylaxis in the prevention of human rabies

Rabies is one of the most feared zoonotic diseases because it has the highest human case-fatality proportion of all conventional infectious diseases.<sup>13</sup> This neglected disease is caused by several ribonucleic acid viruses in the family *Rhabdoviridae*, genus *Lyssavirus*. Although all lyssaviruses cause rabies, the most significant member of the genus is rabies virus (RABV). Only a few cases of survival have been documented, and this acute progressive encephalitis is considered incurable.<sup>14,15</sup> Globally, rabies occurs in more than 150 countries and territories. More than 3 billion people live in areas in which the disease is enzootic (Figure 1).<sup>16</sup> Worldwide, millions of exposures are registered, resulting in tens of thousands of human deaths, with most occurring in Asia and Africa, despite the availability of safe and efficacious biologics. Of this burden, 30%–60% of the victims of dog bites are children



**Figure 1** Global prevalence of rabies. Four categories of countries or areas, from those at no risk to those at low, moderate and high risk.

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under the age of 15 years.<sup>17</sup> Historically, vaccination forms the cornerstone of a multipronged approach to rabies prevention. Interestingly, rabies differs from most other vaccine-preventable infectious diseases, because modern intervention permits prevention both before exposure (pre-exposure prophylaxis) and after exposure (postexposure prophylaxis [PEP]). Vaccination involves the use of inactivated rabies vaccines derived either from cultured cells (human diploid cells, primary chick embryo cells, and Vero cells) or avian embryo cells (duck). Annually, more than 15 million people worldwide are estimated to receive PEP to prevent the disease, which is estimated to prevent

hundreds of thousands of rabies deaths.<sup>18</sup> Based on the types of interaction with suspected rapid animals, exposure is broadly classified into three categories viz. I, II, and III (Table 3).<sup>19</sup> Used promptly and appropriately, modern rabies vaccines are highly effective in the rapid induction of virus-neutralizing antibodies. Understandably, vaccination alone is inadequate to protect rabies victims from all animal bite contacts, especially associated with multiple and severe bites.<sup>20</sup> Based upon viral dose and route, even accelerated vaccination schedules may be inadequate after severe exposures.<sup>21</sup> Today, most people die of rabies because of a lack of proper education about the disease and access to

**Table 3** Categories of rabies contacts for consideration of prophylaxis

Category	Types of contact	PEP	Remarks
I	Touching or feeding of animals; licks on the skin	None	Not considered exposure.
II	Nibbling of uncovered skin; minor scratches or abrasions without bleeding; licks on broken skin	Wound cleaning and vaccination	Unvaccinated subjects: vaccine: IM doses of 1 mL or 0.5 mL given as four to five doses over 2–4 weeks. Vaccinated subjects: vaccine: IM doses of 1 mL or 0.5 mL, or ID given at 0.1 mL, given as two doses separated by 3 days.
III	Single or multiple transdermal bites or scratches; contamination of mucous membrane with saliva from licks; exposure to bat bites or scratches, even if not evident	Wound cleaning, vaccination, and infiltration of RIG	Unvaccinated subjects: vaccine: IM doses of 1 mL or 0.5 mL, or ID at 0.1 mL, given as four to five doses over 2–4 weeks. RIG: HRIG at 20 IU/kg or ERIG at 40 IU/kg. Vaccinated subjects: vaccine: IM doses of 1 mL or 0.5 mL, or ID 0.1 mL, given as two doses separated by 3 days.

**Abbreviations:** ERIG, equine rabies immunoglobulin; HRIG, human rabies immunoglobulin; IM, intramuscular; PEP, postexposure prophylaxis; RIG, rabies immunoglobulin; ID, intradermal.

affordable, lifesaving biologics. Occasional PEP failures may occur, in part due to lack of timely administration of RIG, in concert with vaccination.<sup>22,23</sup> When PEP is administered in a timely and appropriate manner, RABV may be cleared before a productive infection of the central nervous system manifests.<sup>24</sup> Although protective immunity against lyssaviruses is deemed to be complex and due to a suite of factors, humoral immunity directed against the outer viral G is felt to be paramount. The mode of protection is likely to be a combination of local virus neutralization by antibodies or via antibody-mediated clearance of virus-infected cells.<sup>25</sup> Because the bite of a rabid or suspected rabid animal is presumed to have virus excreted in the saliva, PEP includes immediate local treatment of all bite wounds and scratches with thorough washing and disinfection, local wound infiltration with RIG, and vaccination. This combination of active and passive immunization is considered the status quo for PEP, except for those persons who have been previously immunized with a rabies vaccine via a recognized schedule or a documented adequate RABV antibody titre.<sup>26</sup> The aim of passive immunization using RIG is to confer short-lived immunity characterized by rapid onset and lack of immunological memory, while the goal of active immunization using rabies vaccine is to elicit durable immunity characterized by delayed onset and immunological memory.<sup>27</sup> Understandably, the antibodies administered passively compensate for the time necessary for vaccine-induced antibodies to appear. Hence, the RIG should be given usually through the seventh day after the first dose of vaccine is administered. However, beyond the seventh day, RIG is not indicated, as an active antibody response to cell culture rabies vaccine is presumed to have occurred.<sup>28</sup> Although the combination of rabies vaccine and RIG is nearly 100% effective in prevention before illness, attempts to use rabies vaccine or RIG after the onset of symptomatic rabies have not been proven beneficial.<sup>29</sup>

## Rabies immunoglobulin – polyclonal

Historically, polyclonal RIG has been used for PEP of human rabies since the mid-20th century. The polyclonal RIG is relatively easy to produce, possesses high potency, a broad spectrum of virus-neutralizing activity, polyspecificity that prevents the selection of neutralization escape mutants, and multiple effector functions, mediated by several isotypes, due to its heterogeneous nature.<sup>30</sup> Typically, manufacture involves purification of immunoglobulin (Ig)G from the plasma of immune human or animal donors, such as horses (equine rabies immunoglobulin [ERIG]). Both ERIG and

human rabies immunoglobulin (HRIG) are currently licensed for use in humans and widely used in developing and developed countries, respectively. The ERIG is a heterologous molecule and highly immunogenic in humans, resulting in induction of human antiequine antibody responses, leading to rapid clearance of ERIG, necessitating higher dosing and culminating in induction of severe type III hypersensitivity reactions and serum sickness, which is sometimes fatal.<sup>31</sup> Consequently, often, physicians are hesitant to use ERIG, thus providing incomplete PEP, which may result in failures.<sup>32</sup> These adverse events are essentially due to the Fc region of the Ig, and hence ERIG devoid of the Fc region: ie, F(ab')<sub>2</sub>, which is obtained by pepsin digestion of IgG, is a format currently in use.<sup>33</sup> The F(ab')<sub>2</sub> of ERIG per se retains effectiveness because of its ability to bind to the target mediated by Fab region, which is a prerequisite for neutralization and elimination of the pathogen. Nevertheless, the ERIG is known to pose some risks to humans, which may be mitigated to some extent by improved purification processes.<sup>33</sup> Medical concerns may be managed by performing skin tests or by premedication with antihistamine and corticosteroids.<sup>34,35</sup> Compared with HRIG, the less expensive nature of ERIG seems to be the primary reason for its continued use in developing countries. All licensed RIGs are expected to neutralize all known RABV variants, but no available product will neutralize all described lyssaviruses.<sup>36</sup>

The quest for an improved alternative to heterologous animal serum products resulted in the initial development of HRIG, which is its homologous equivalent. Modern HRIG is safe, nonimmunogenic, and well tolerated by humans. The incidence of anaphylaxis or serum sickness is virtually unknown.<sup>37</sup> However, as a human plasma product, it has the potential for transmission of blood-borne infectious agents, which can be mitigated by treatment with solvents or detergents<sup>38</sup> or heat treatment.<sup>39</sup> Such processes are expensive and not generally affordable in developing countries, where canine rabies remains the primary public health problem.<sup>40</sup> The worldwide inaccessibility of HRIG and its high cost of production place it out of reach of most patients in the developing world.<sup>31</sup> Nevertheless, HRIG has largely replaced ERIG in several countries, and future animal welfare concerns may further limit the availability of ERIG. With the idea of exploiting certain desirable qualities of polyclonal RIG and to overcome the limitations of existing ERIG and HRIG in parallel, RIG production has been reported originating from other species, including chickens,<sup>41</sup> rabbits,<sup>42</sup> and sheep.<sup>43</sup> It remains to be seen whether polyclonal RIG from these species would be viable for clinical use. Regardless of

the species of origin, polyclonal RIG in general has certain constraints, including the need for donor recruitment and immunization; multiple inoculations and bleeding procedures; donor retention; ethical problems; lower specific activity, which may necessitate the use of more protein, which may lead to higher viscosity and adverse events; variable batch-to-batch consistency; supply limitations from competing use of plasma products; and the potential risk of transmission of infectious agents. For such economic, supply, and safety reasons, replacement of HRIG and ERIG is advocated, and the World Health Organization strongly encourages development of alternative products.<sup>44</sup>

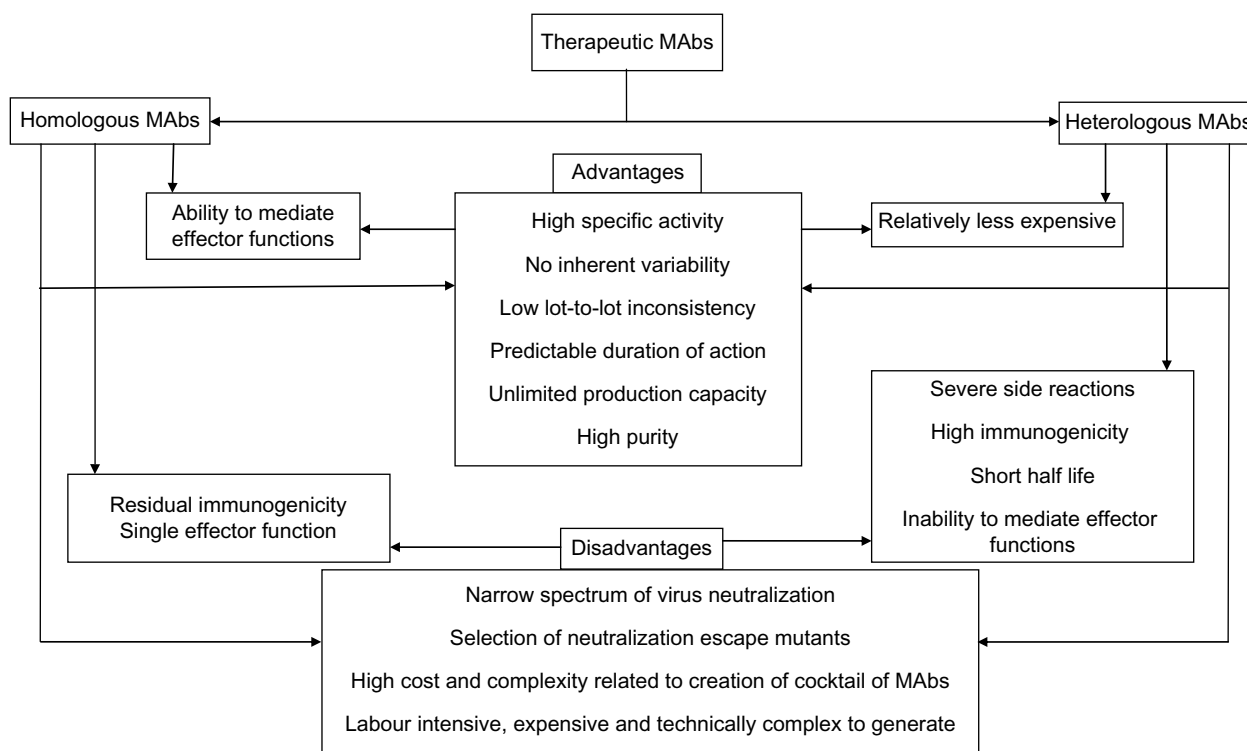
## Alternatives to polyclonal immunoglobulins

The invention of the concept of MAb technology by Kohler and Milstein<sup>45</sup> in 1975 revolutionized biomedicine and has become a billion-dollar industry. For the first time, researchers and clinicians were able to replicate and harness the therapeutic power of single antibodies created by the immune system.<sup>46</sup> Clearly, MAbs have contributed immensely to the fields of basic research and disease diagnosis. The versatility of MAbs for *in vitro* applications prompted scientists to explore their usefulness for therapeutic applications, which resulted in the launch of the first US Food and Drug Administration-approved mouse MAb (Orthomab OKT3) for treating acute organ transplantation complications.<sup>47</sup> The use of hybridomas in MAb production enables a sustained production of antibody and is not dependent on the life of the donor host as with polyclonal antibody production.<sup>48</sup> The rationale behind using MAbs for therapy is that they provide a more potent product with better activity than their polyclonal counterparts.<sup>49</sup> Additionally, they do not seem to have the inherent variability with regards to epitope and isotype,<sup>30</sup> and are homogeneous in nature and hence exhibit relatively low lot-to-lot variability. Significantly, the duration of action of MAbs is predictable and likely to be related to the biological half-life.<sup>50</sup> High specific activity of MAbs essentially makes administration of a low amount of protein and volume possible, which per se avoids several adverse events, including the concern for compartment syndrome. Although MAbs have significant promise as therapeutic agents, they are not without limitations: eg, 1) they may be expensive; 2) by definition, they target a single epitope and hence provide one type of effector function corresponding to their isotype; 3) although the specificity of MAbs is a strength, a pathogen that possesses rapid antigenic variation poses a significant hurdle

for broader MAb development; and 4) they may select for neutralization escape variants as a result of microbial mutation or microevolution.<sup>51</sup> The use of MAbs that target conserved areas of viral particles, or a cocktail of MAbs that target various epitopes, can obviate this concern.<sup>30,52</sup> In fact, the World Health Organization has advocated the use of antirabies MAb cocktails for rabies PEP and does not recommend the use of single MAbs, due to the potential of viral escape.<sup>44</sup> However, this approach of using a cocktail of MAbs would also have the drawback of increasing the cost of production and the complexity of regulatory issues involving their efficacy and safety (Figure 2).<sup>53</sup>

## Native – mouse MAbs – full length

During the last 3 decades, numerous murine MAbs against the RABV G that neutralize RABV and other lyssaviruses, both *in vitro* and *in vivo*, have been developed and extensively characterized by several groups worldwide.<sup>54–62</sup> They are specific to one of five distinct antigenic sites on the RABV G (antigenic sites I, II, III, IV, and minor site a),<sup>56,61,63,64</sup> with the vast majority of them recognizing either antigenic site II or III (Figure 3).<sup>65</sup> Antigenic site II is discontinuous and conformation dependent,<sup>59</sup> whereas antigenic site III is predicted to be continuous and conformation dependent.<sup>61</sup> No single MAb will neutralize all known RABV variants,<sup>67</sup> so MAbs can be broadly neutralizing only when used in combination,<sup>60,68,69</sup> which is not the case when used alone, because the G is prone to a high level of diversity in nature. Besides neutralization activity in cell culture, MAbs directed against the RABV G have also been shown to protect Syrian hamsters from lethal challenge.<sup>32,56,60</sup> Cocktails of mouse MAbs have been envisioned to be less expensive alternatives to polyclonal RIG for PEP to prevent rabies in humans, because they performed as well as HRIG in animal models (Table 4).<sup>70,71</sup> Initially, the first generation of mouse-derived MAbs suffered side effects due to an unwanted immune reaction in humans, referred to as a “human antimouse antibody” response.<sup>72,73</sup> This response, characterized by fever, chills, arthralgia, and life-threatening anaphylaxis, was similar to the serum sickness observed several decades earlier with animal antisera.<sup>46</sup> Instability of some murine hybridomas, immunogenicity, potential short half-life, and contamination with potential pathogen of murine MAbs pose both scalability and safety risks that essentially constrain their use in human therapy. The full promise of murine MAbs could be realized by engineering to “humanize” murine antibodies to make them less immunogenic and safer.<sup>46</sup> To this end, a chimeric mouse–human



**Figure 2** Advantages and disadvantages of therapeutic monoclonal antibodies (MAbs).

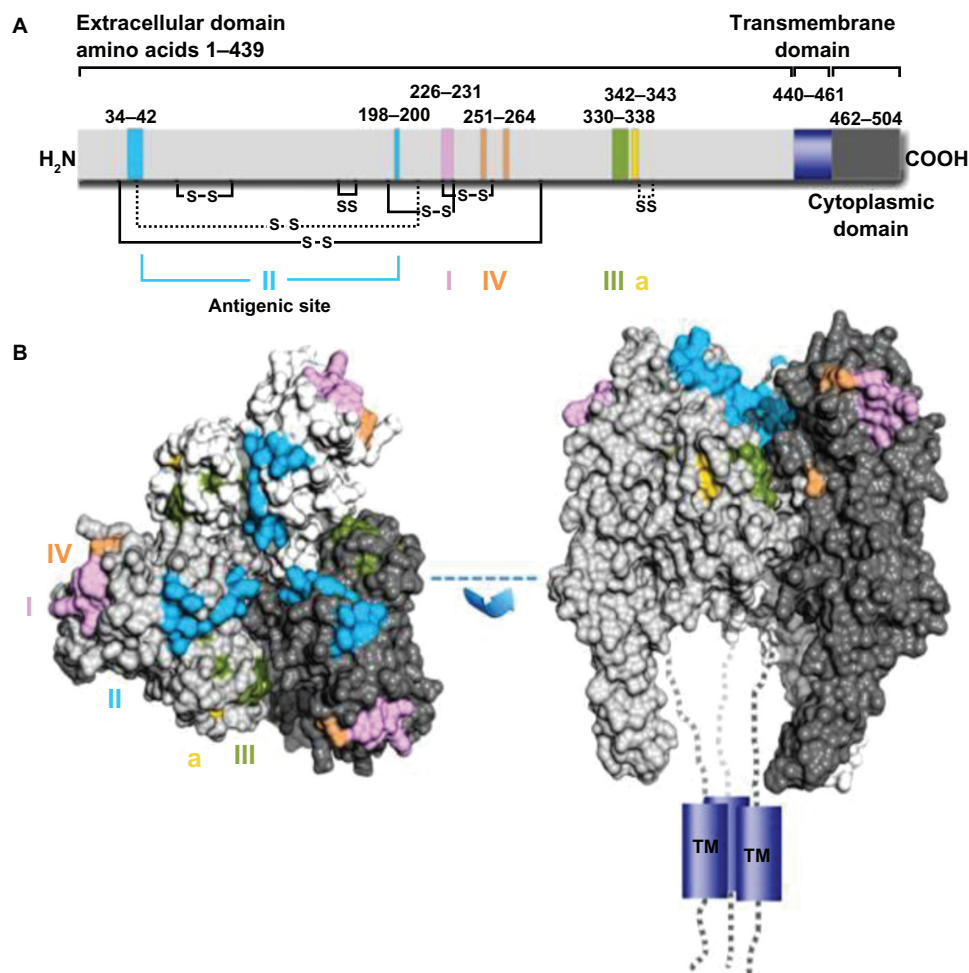
version of MAb 62-71-3 was expressed in tobacco leaves and found to be an appropriate candidate MAb in the making of a novel antibody cocktail.<sup>74</sup>

## Native – human MAbs – full length

Human MAbs are nonimmunogenic molecules that essentially retain all the desirable qualities of murine MAbs. They are ideally suited for prophylaxis because they undergo affinity maturation *in vivo* and represent the natural Ig repertoire.<sup>75</sup> The development of human MAbs by hybridoma technology was difficult at first due to poor accessibility to primed human B cells and a lack of ideal myeloma fusion partners.<sup>76</sup> However, human B-cell immortalization could be accomplished by employing myeloma or heteromyeloma fusion partners and Epstein–Barr virus (EBV), although with varying levels of efficiency and clonal instability.<sup>77</sup> Interestingly transgenic mice expressing a human antibody repertoire allowed the creation of human MAbs through the development of murine hybridomas in a relatively simpler manner.<sup>78</sup> Prior work showed that EBV-transformed cell lines often secrete low amounts of antibodies that are of the IgM class, and that human MAbs so derived may not be suitable for biomedical applications due to the presence of EBV antigens.<sup>79</sup> This problem could be overcome by stable and high-efficiency expression of recombinant human MAbs in heterologous or

homologous systems such as Chinese hamster ovary (CHO) and human retinal (PER.C6) cell lines, respectively.<sup>80,81</sup>

Several RABV-neutralizing human MAbs were established by using the human x mouse heterohybridoma method,<sup>64,79,82</sup> EBV transformation,<sup>65,80</sup> phage display technology,<sup>83</sup> and transgenic mouse technology.<sup>84</sup> Two human MAbs viz CR57 (antigenic site I) and CR4098 (antigenic site III) recognize nonoverlapping, noncompeting epitopes, with broader neutralization of street RABV isolates when used as a combination, displaying *in vitro* neutralization of all RABV tested so far and demonstrating an efficacy profile similar to HRIG in animal studies (Table 4).<sup>69,83,85</sup> A single antigenic site III specific human MAb RAB1 derived from transgenic mouse (Medarex, Inc, Princeton, NJ, USA) has been shown to efficiently neutralize many currently identified RABV isolates except a fixed RABV: ie, CVS-11, and a bat RABV from Peru *in vitro*,<sup>67</sup> and protect Syrian hamsters from lethal challenge (Table 4).<sup>84</sup> Similarly, an antigenic site II specific human MAb No 254 created through EBV transformation of human B cells exhibited a broad spectrum of RABV neutralization and has been shown to be as effective as ERIG in an experimental animal model.<sup>80</sup> A human MAb will be of value to the majority of people only if it can be produced in large amounts for a cost comparable with the cost of current ERIG but lower than HRIG products. Initial



**Figure 3** Representation of antigenic sites on the mature rabies virus glycoprotein G.

**Notes:** (A) Linear schematic showing the relative position and amino acid numbering of the antigenic sites (I, II, IV, and a) within the extracellular domain of G. The numbering relates to the mature glycoprotein (after removal of the 19-mer signal peptide). The position of disulfide bridges has been indicated based on an alignment with G of vesicular stomatitis virus (VSV) (PDB ID 2J6J). (B) Top (left) and side view (right) of a surface rendering of the homotrimeric prefusion structure of VSV G. Monomers are shown in shades of grey. Rabies antigenic sites, highlighted in color as in (A), were superpositioned based on sequence alignment with VSV (~20% sequence identity). Portions of the protein for which no three-dimensional structural information was available are indicated as dotted lines or, if predicted to be transmembrane regions, as cartoon barrels.

**Figure 3A** - Copyright © American Society for Microbiology. Adapted with permission from *J. Virol.* 2005;79(8):4672–4678. Marissen WE, Kramer A, Rice A, et al. Novel Rabies Virus-Neutralizing Epitope Recognized by Human Monoclonal Antibody: Fine Mapping and Escape Mutant Analysis.<sup>123</sup>

**Abbreviations:** COOH, carboxyl terminus; H<sub>2</sub>N, amino terminus; SS, disulfide bridge; TM, transmembrane.

industrial-level scale-up of heterohybridoma cell lines may not be cost-effective because of instability and low levels of antibody production.<sup>79</sup>

## Homologous products (recombinant) – fully human antibody fragments

An early focus of antibody engineering was on reducing the immunogenicity of rodent antibodies via chimerization

and humanization. This focus later expanded to include engineering for enhancing several other desirable traits.<sup>86</sup> Phage and ribosomal display technologies for discovery and selection of antibodies in vitro are less time-consuming and highly suitable. The antibodies are usually displayed as Fab (VH–CH, VL–CL)<sup>87</sup> and scFv (VH–VL) fragments.<sup>88</sup> There are several reports on isolation of human scFvs specific for RABV G by phage display<sup>89</sup> and ribosome display,<sup>90</sup> and Fabs

**Table 4** In vivo efficacy data of antirabies monoclonal antibodies (MAbs) in simulated postexposure prophylaxis models

MAb	Animal	MAb dose	Rabies vaccine	Survival (%)	Reference
CL184 (CR57/CR4098)	Hamster	20 IU/kg	Administered	100	69
62-7-13/M777	Hamster	200 IU/kg <sup>a</sup>	Not administered	100	70
17C7	Hamster	21 IU/kg	Administered	95	84

**Note:** <sup>a</sup>Assumed a hamster body weight of 0.1 kg; 0.05 mL of 400 IU/mL was administered.

by phage display<sup>91</sup> and conversion of scFv.<sup>92</sup> The scFvs are relatively less stable and low in half-life than the Fab, due to their lower molecular weight.<sup>93</sup> A Fab displayed better tissue penetration and efficacy sufficient to replace HRIG when administered with nanoparticles (Fab094–CPNPs).<sup>91</sup> Nevertheless, antibody fragments may hold promise as a potential alternative to full-length human MAbs due to their simpler production process and lower production costs.<sup>94</sup> However, they are not as ideal as HRIG when it comes to mediation of viral clearance through antibody-dependent cellular cytotoxicity and will have much lower half-life compared with full antibodies. Presumably, the antibody fragments may be suitable for PEP if they are appropriately engineered. To this end, an interesting derivative of scFv, an scFv–Fc (scFv–hinge–CH2–CH3) fusion protein expressed in yeast, was found to mediate RABV neutralization<sup>95</sup> and effector functions.<sup>96</sup>

## Homologous products (recombinant) – fully human intact IgG

Important parameters that drive selection of the most optimal systems for expression of antibodies include basic structure, protein folding and glycosylation,<sup>97</sup> productivity, ease of purification,<sup>98</sup> product quality and quantity, safety issues, time to clinic and market, and economic considerations such as cost of goods and regulatory issues.<sup>99</sup> In general, eukaryotic systems such as mammalian cells,<sup>99,100</sup> insect cells,<sup>97</sup> yeast,<sup>101</sup> plants,<sup>74,102</sup> green algae,<sup>103</sup> and transgenic animals<sup>104</sup> hold the greatest promise for expressing recombinant human MAbs. However, mammalian cells are predominantly used because they can correctly fold, assemble, glycosylate, and secrete antibodies.<sup>105</sup> It is possible to achieve yields in excess of 3 g/L using optimized cell culture processes. However, development of a stable and high-producing cell line is quite challenging and is a critical step in the development of biotherapeutics.<sup>106</sup>

Several mammalian cells, namely CHO, mouse myeloma (NSO, Sp2/0), baby hamster kidney (BHK-21), human embryonic kidney (HEK-293), and PER.C6 cells, have gained regulatory approval for production of recombinant proteins. The CHO cell line, in particular, has become the workhorse for industrial manufacture, and has even surpassed some microbial systems in productivity.<sup>107</sup> However, it has certain limitations, such as the need for gene amplification and the selection pressure that is considered responsible for instability of expression.<sup>108</sup> Interestingly, a recombinant rhabdovirus-based vector suitable for rapid and cost-effective industrial-scale production of antibody, which utilizes the CHO cell line as a substrate, has been reported.<sup>109,110</sup> A human origin cell line PER.C6 has been developed as an alternative to the CHO cell line for large-scale manufacturing of recombinant human MAbs.<sup>100</sup> It can be adapted to grow under different conditions, produces a high level of recombinant proteins in a stable manner, does not require gene amplification, and does not add nonhuman glycan structures to proteins.<sup>81</sup> The choice for a certain cell expression system will have to take into account the production cost in relation to the target population, which for many neglected diseases is disproportionately the poor people living in developing countries.<sup>111</sup> Hence, in some instances, it may be beneficial to explore inexpensive expression systems such as yeast<sup>112</sup> and plants.<sup>113</sup>

## Clinical trials

Understandably, human clinical trials for new types of rabies biologics are not easy to accomplish today. In general, a clinical study involves applied research using human volunteers that is intended to add to medical knowledge related to the treatment, diagnosis, or prevention of diseases or conditions, in this case a fatal viral zoonosis. Historically, there are two main types of such studies: 1) actual clinical trials and 2) observational studies, such as occurred with the introduction of RIG and the human diploid cell vaccine.

**Table 5** Human anti-rabies virus MAbs that have passed through Phase I/II clinical trials

Developing agency	Licensing agency	Human MAb	Antigenic site specificity(ies)	Isotype(s)	Technology platform(s)		Clinical trials registry
					Native human MAbs	Recombinant human MAbs	
Crucell Holland BV, the Netherlands	Crucell Holland BV, the Netherlands	CR57	I	IgG1	Human x mouse heterohybridoma	PER.C6 cell line	NCT00708084 <sup>119</sup> NCT00656097 <sup>120</sup> NCT01228383 <sup>121</sup>
		CR4098	III	IgG1	Phage display technology	PER.C6 cell line	
Massachusetts Biologicals Limited, USA	Serum Institute of India Ltd, India	17C7	III	IgG	Transgenic mouse	CHO cell line	CTRI/2009/091/000465 <sup>122</sup>

**Abbreviations:** CHO, Chinese hamster ovary; Ig, immunoglobulin; MAbs, monoclonal antibodies.



In a clinical trial, human volunteers receive specific interventions according to a protocol created by the investigators. These interventions may be medical products such as drugs or devices. The investigators try to determine the safety and efficacy of the intervention by measuring certain outcomes in the participants. Such clinical trials in drug development are described by various phases.<sup>114</sup> These include Phase 0 (exploratory study), Phase I (safety), Phase II (effectiveness), Phase III (safety and effectiveness), and Phase IV (postmarketing safety, efficacy, and optimal use).<sup>115</sup> To date, one anti-RABV human MAbs (SII Rmab) derived from an engineered CHO cell line has successfully passed through a Phase I clinical trial<sup>116</sup> (Table 5), and a Phase II clinical trial has been initiated very recently.<sup>117</sup> Similarly, a combination of two anti-RABV human MAbs, called CL184, produced using the innovative MAbstract technology and PER.C6 cell line has successfully progressed through Phase I and Phase II clinical trials (Table 5).<sup>118</sup> Phase III clinical trials are expected to be conducted in the near future. For the first time in history, these products may be launched globally and eventually reach the clinic sooner rather than later. It is tempting to speculate that human MAbs will slowly replace the polyclonal RIGs that are currently in use and over the next several years slowly dominate the market place. Considering the slow evolution of rabies biologics over the past century, a period of more than 3 decades of such research for such a major paradigm shift to occur for effective PEP of humans with MAbs seems well worth the wait.

## Disclosure

The authors report no conflicts of interest in this work.

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