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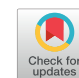
Biosafety and Health 1(1) (2019) 6–13

Contents lists available at ScienceDirect

Biosafety and Health

journal homepage: www.elsevier.com/locate/bsheal

Virus inoculation and treatment regimens for evaluating anti-filovirus monoclonal antibody efficacy *in vivo*



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ARTICLE INFO

Article history:
Received 12 November 2018
Received in revised form 7 January 2019
Accepted 21 February 2019
Available online 1 March 2019

Keywords:
Filovirus
Ebola virus
Marburg virus
Monoclonal antibodies
Therapeutics
Animal model

ABSTRACT

The development of monoclonal antibodies to treat disease caused by filoviruses, particularly Ebola virus, has risen steeply in recent years thanks to several key studies demonstrating their remarkable therapeutic potential. The increased drive to develop new and better monoclonal antibodies has necessarily seen an increase in animal model efficacy testing, which is critical to the pre-clinical development of any novel countermeasure. Primary and secondary efficacy testing against filoviruses typically makes use of one or more rodent models (mice, guinea pigs, and occasionally hamsters) or the more recently described ferret model, although the exact choice of model depends on the specific filovirus being evaluated. Indeed, no single small animal model exists for all filoviruses, and the use of any given model must consider the nature of that model as well as the nature of the therapeutic and the experimental objectives. Confirmatory evaluation, on the other hand, is performed in nonhuman primates (rhesus or cynomolgus macaques) regardless of the filovirus. In light of the number of different animal models that are currently used in monoclonal antibody efficacy testing, we sought to better understand how these efficacy tests are being performed by numerous different laboratories around the world. To this end, we review the animal models that are being used for antibody efficacy testing against filoviruses, and we highlight the challenge doses and routes of infection that are used. We also describe the various antibody treatment regimens, including antibody dose, route, and schedule of administration, that are used in these model systems. We do not identify any single best model or treatment regimen, and we do not advocate for field-wide protocol standardization. Instead, we hope to provide a comprehensive resource that will facilitate and enhance the continued pre-clinical development of novel monoclonal antibody therapeutics.

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1. Introduction

Filoviruses comprise a relatively small family of negative sense RNA viruses that have a disproportionately large impact on global public health and biosecurity (Table 1). The most infamous member of this family is Ebola virus (EBOV), which has been responsible for causing sporadic outbreaks of severe disease mostly in Central Africa, at least since its discovery in 1976 [1,2]. However, the related but less renowned filoviruses, Sudan virus (SUDV), Bundibugyo virus (BDBV), Marburg virus (MARV), and Ravn virus (RAVV), have also been implicated in causing numerous, severe outbreaks throughout Africa [1,2]. Conversely, Taï Forest virus (TAFV) has only been implicated in a single, non-fatal case of disease in humans, while Reston virus (RESTV) appears not to cause any symptomatic illness in humans [3]. Two other recently-described filoviruses, Bombali and Lloviu virus (BOMV and LLOV, respectively), have yet to be isolated and their pathogenic potential therefore remains unknown [4,5].

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Although many of the outbreaks caused by the human-pathogenic filoviruses have been deadly, with case fatality rates approaching 90% in some instances, the vast majority have been small in size—involving <400 cases—and easily controlled [1,2]. The exception came in 2013, however, when EBOV was found for the first time in Western Africa, where it incited an epidemic that lasted over two years, infected nearly 30,000 people (resulting in over 11,000 deaths), and devastated the countries of Liberia, Guinea, and Sierra Leone [6]. The unprecedented nature of this outbreak not only brought EBOV to the forefront of the world's attention, but it also served as a painful reminder that, at the time, no clinically-licensed countermeasure existed to treat or prevent filovirus disease.

To date, there remains no vaccine or therapeutic that has been unconditionally approved for clinical use against filoviruses, although considerable effort has been invested in the discovery and pre-clinical development of novel and effective countermeasures, particularly in the wake of the West African outbreak [7]. Among the most promising therapeutic candidates to arise in the last several years are the monoclonal antibody-based treatments targeting the filovirus glycoprotein (GP), the sole viral protein responsible for virus attachment/entry and the only viral protein found on the surface of the virion. The utility of monoclonal antibodies as

https://doi.org/10.1016/j.bsheal.2019.02.004
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Table 1
Filoviruses and pathogenicity.

Family	Genus	Species	Virus	Virulence in humans[a]
Filoviridae	*Ebolavirus*	*Zaire ebolavirus*	Ebola virus (EBOV)	++++
		Sudan ebolavirus	Sudan virus (SUDV)	+++
		Bundibugyo ebolavirus	Bundibugyo virus (BDBV)	++
		Taï Forest ebolavirus	Taï Forest virus (TAFV)	+
		Reston ebolavirus	Reston virus (RESTV)	−
		Bombali ebolavirus	Bombali virus (BOMV)	?
	Marburgvirus	*Marburg marburgvirus*	Marburg virus (MARV)	++++
			Ravn Virus (RAVV)	+++
	Cuevavirus	*Lloviu cuevavirus*	Lloviu virus (LLOV)	?

[a] Virulence is estimated based on a variety of factors, including case fatality rates, outbreak sizes, and virulence in nonhuman primates, with "++++" highly virulent and "−" not virulent.

an effective therapeutic against filovirus disease was unequivocally demonstrated in 2012, when Qiu et al. published a report detailing the reversion of severe EBOV disease in five nonhuman primates (NHPs) following treatment on day 5 post-infection with ZMapp, a cocktail of three EBOV GP-specific monoclonal antibodies [8]. This work represented the culmination of several prior successful studies [9–13], and it refuted the historical impression that passive antibody therapy for filoviruses could not succeed—a notion based primarily on early, unsuccessful treatments using convalescent blood [14], polyclonal equine immunoglobulin [15,16], and the anti-EBOV monoclonal antibody KZ52 [17]. Since 2012, the number of monoclonal antibodies and antibody cocktails that have been developed to treat filovirus infections has exploded [7], with the field edging consistently closer to achieving the goal of a pan-filovirus therapeutic effective with a single dose administered even after symptoms of disease have developed.

The remarkable success of monoclonal antibody-based therapeutics against filoviruses has been facilitated, at least in part, by the various animal models that are used for efficacy testing (Table 2) [18–20]. Mice, guinea pigs, and (less commonly) hamsters have all been used for primary efficacy screening of monoclonal antibody therapeutics against EBOV, SUDV, MARV, and RAVV, while the ferret model has been used for efficacy screening against EBOV and SUDV, as well as BDBV, for which no rodent model exists. NHPs represent the gold-standard model for filovirus countermeasure evaluation [21], and as such, they have been reserved for confirmatory evaluation of a candidate antibody or antibody cocktail against EBOV, SUDV, BDBV, and MARV. Notably, although a number of monoclonal antibodies have so far been developed—at least one for every pathogenic filovirus—the majority of the *in vivo* efficacy data has been obtained against EBOV.

In an effort to promote the additional development and pre-clinical evaluation of anti-filovirus countermeasures, we have collated data from a number of studies investigating the efficacy of monoclonal antibody therapeutics. Herein, we review the animal models that are used for antibody efficacy testing against various filoviruses and highlight the various challenge doses and routes of infection that are routinely used (See Box). Moreover, we describe the antibody treatment regimens that are used in these animal models, including antibody dose, as well as route and schedule of administration. Based on this comprehensive technical review, we hope to provide a resource for the field to consult when designing future monoclonal antibody efficacy experiments.

Box: Quantification of filoviruses.

There is no single, standardized method used among all laboratories to quantify filoviruses, and for this reason, it can be difficult to compare virus titers (and therefore inoculation doses) among different experiments from different groups. In general, the two most common quantification methods are the plaque assay and the endpoint dilution assay [22]. The plaque assay relies on the direct enumeration of viral plaques counted across several cell monolayers infected with serially-diluted virus, and the results are expressed as a viral titer in plaque forming units per ml (PFU/ml). An alternative, but closely related, method uses immunofluorescence to count viral foci (rather than plaques), and these results are expressed in focus forming units per ml (FFU/ml). The most common endpoint dilution assay is the 50% tissue culture infective dose ($TCID_{50}$) assay, which is performed by counting the number of wells displaying cytopathic effect after infection with serially-diluted virus. The results are expressed as $TCID_{50}$/ml and reflect the amount of virus required to infect 50% of cells in a given culture. A similar endpoint dilution assay can be performed using groups of animals infected with serially-diluted virus to determine the dose of virus that is lethal in 50% of infected animals (LD_{50}), and these results are expressed as LD_{50}/ml. Notably, however, this method of virus quantification is ethically and practically permissible only for rodent models of infection. While it is generally accepted that the $TCID_{50}$ assay produces a titer that is tenfold higher than the plaque assay for EBOV infection, comparisons between different quantification methods have only been published for a few filoviruses [22,23]. In many cases, the precise relationship between the titers calculated from different quantification methods is not known, and, because this relationship may vary depending on the specific virus variant, cell line, and methodology used, it may not be universally applicable from one study to another. In this review, we have endeavoured to provide as much information as possible regarding filovirus inoculation doses; however, reporting a single, consistent unit for all studies discussed here is not possible.

2. Mice

Laboratory mice are the most commonly used animal model in filovirus research [18], and not surprisingly, they are frequently used for the primary evaluation of monoclonal antibody efficacy against certain filoviruses (Supplementary Table 1). Indeed, mice are inexpensive, easy to handle, and well characterized, making them the first choice for initial screening of a variety of therapeutics and vaccines [18]. However, because wildtype filoviruses do not cause disease in immunocompetent mice, mouse-adapted virus variants or immunodeficient mouse strains are required. Mouse-adapted variants of EBOV (MA-EBOV), MARV (MA-MARV), and RAVV (MA-RAVV) have all been described, and each recapitulates many key hallmarks of filovirus disease [18]. All of these viruses have been used for monoclonal antibody efficacy testing, most commonly in immunocompetent BALB/c mice and occasionally in C57BL/6 mice (Table 2 and Supplementary Table 1) [12,13,24–43]. To date, a mouse-adapted SUDV has not been described, and as a result, efficacy testing against this virus has relied on immunodeficient mice, such as $IFNAR^{-/-}$ or STAT1 KO mice [26–32,34–36,43–46], which are susceptible to disease caused by wildtype SUDV despite the fact that uniform lethality is not routinely observed [47,48]. Likewise, wildtype EBOV and MARV are occasionally used in $IFNAR^{-/-}$ mice for antibody efficacy

Table 2
Animal models used for monoclonal antibody efficacy testing.

		EBOV			SUDV		BDBV	MARV				RAVV		
		WT	MA	GPA	WT	GPA	WT	WT	MA	GPA	HA	WT	MA	GPA
Immunocompetent Mice	BALB/c		X						X				X	
	C57BL/6		X											
Immunodeficient Mice	IFNAR$^{-/-}$	X			X			X						
	STAT1 KO				X									
Guinea Pigs	Hartley			X		X				X				X
	Strain 13			X										
Hamster	Syrian Golden										X			
Ferrets	Domestic	X			X		X							
Macaques	Rhesus	X			X		X	X				X		
	Cynomolgus	X					X							

WT, wildtype; MA, mouse-adapted; GPA, guinea pig-adapted; HA, hamster-adapted. X indicates that a given model system has been used to perform monoclonal antibody efficacy testing against the indicated virus. Color gives an indication of the number of studies that have been performed, with red indicating ≥14, orange 9–13, light orange 3–8, and yellow ≤2.

testing [34,49]. BDBV, on the other hand, causes no clinically obvious disease in immunodeficient mice and no mouse-adapted variant has been described, so primary efficacy testing against this virus requires ferrets (described below), which are the only small animals susceptible to lethal BDBV infection [50,51]. Altogether, the vast majority of monoclonal antibody efficacy testing against any filovirus has relied on BALB/c mice and MA-EBOV, although immunodeficient mice used in conjunction with SUDV, in particular, also make up a large proportion of efficacy studies (Table 2 and Supplementary Table 1). Relatively few studies have been performed in mice using the marburgviruses, owing mainly to the lack of candidate monoclonal antibodies.

2.1. Inoculation

A critical consideration in the use of the mouse model for efficacy testing against filoviruses is the virus inoculation dose, particularly with respect to MA-EBOV. MA-EBOV was generated nearly twenty years ago through successive passaging of wildtype EBOV (variant Mayinga) in suckling mice until a virus isolate capable of causing uniform lethality in immunocompetent mice (BALB/c, C57BL/6, and CD-1) was obtained [52]. Although MA-EBOV has been used extensively since then, many recent studies have reported a lack of uniform lethality in BALB/c mice following intraperitoneal injection of the virus (Supplementary Table 1) [25,53,54]. This absence of uniform lethality can be problematic, particularly when designing experiments to evaluate antiviral countermeasures, which often assume 100% lethality in control-treated animals in order to power the study appropriately with the least number of animals possible. Interestingly, a recent study by Haddock et al. demonstrated that a higher dose of MA-EBOV generally resulted in a decrease in lethality in both BALB/c and C57BL/6 mice [54], a phenomenon that has been observed previously [55]. The reasons for this phenomenon remain unclear, but it has been speculated that a higher viral load may elicit a more robust innate immune response, thus hampering infection, and/or that a higher proportion of defective-interfering particles associated with a high viral load may inhibit productive virus replication, thus reducing disease severity [55]. A comprehensive review of monoclonal antibody efficacy studies revealed a tendency towards increased survival in control mice inoculated with 100 or 1000 PFU/FFU *versus* those inoculated with 1000 LD_{50} (equivalent to approximately 10 PFU) or less (Supplementary Table 1) [12,13,25,29–33,41]. We observed the same trend in a subsequent experiment, wherein BALB/c mice inoculated with higher doses of MA-EBOV (100–10,000 FFU) exhibited partial lethality, while mice inoculated with lower doses (1–10 FFU) exhibited complete lethality (Figure 1). Moreover, the difference between the survival curves at 10 and 1000 FFU was statistically significant ($p = 0.0230$, Log-rank test, GraphPad Prism 7). These data suggest that lower MA-EBOV inoculation doses—within the range of 10 PFU/FFU (or 1000 LD_{50})—may be preferable when designing efficacy studies with BALB/c mice since a lower dose of virus more reliably produces uniform lethality. Additionally, although the majority of monoclonal antibody efficacy studies performed to date have used BALB/c mice, future consideration should be given to using alternate mouse strains, such as CD-1 mice, which exhibit uniform lethality at all inoculation doses above 1 FFU and therefore avoid the problem of viral dose-dependent lethality [54].

Partial lethality also appears to be a feature of some of the mouse models for the remaining filoviruses (Supplementary Table 1). The IFNAR$^{-/-}$ mouse model of SUDV (variant Boniface) infection, in particular, exhibits ~70% lethality at 1000 PFU (Supplementary Table 1) [48], although whether this changes at lower challenge doses is not known. Moreover, different variants of SUDV (such as Gulu) appear to be less pathogenic in IFNAR$^{-/-}$ mice [48], and different immunodeficient mouse strains (such as STAT1 KO mice) appear to be less susceptible to SUDV variant Boniface [47]. Additional work may be required to further optimize the SUDV mouse model; however, in the meantime, efficacy experiments must be designed with partial lethality in mind. Similarly, the mouse-adapted marburgvirus models may not consistently produce 100% lethality [56], although efficacy testing in these model systems is less common, and more work is required. With respect to MARV, in particular, thought should be given to the use of MA-MARV (variant Angola), since this variant is regarded as the most virulent and may potentially offer uniform lethality [57–59].

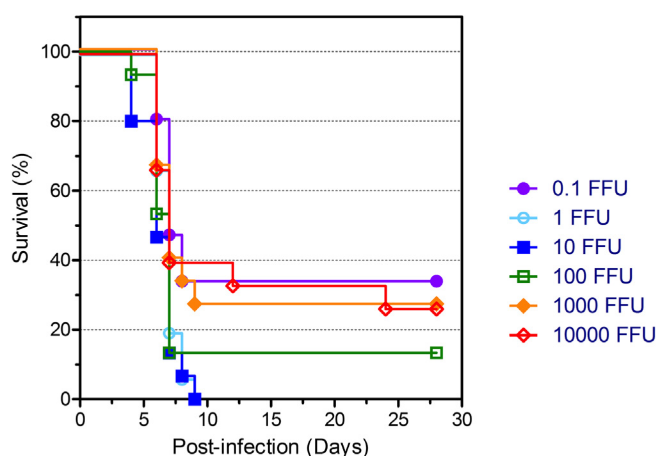

Figure 1. Survival of BALB/c mice infected with varying doses of MA-EBOV. Groups of 15 female, BALB/c mice (aged 6–8 weeks) were inoculated intraperitoneally with 0.1, 1, 10, 100, 1000, or 10,000 focus forming units (FFU) of mouse-adapted (MA) EBOV. Following infection, animals were monitored for 28 days for clinical signs of infection (not shown) and survival, depicted here as a Kaplan-Meier survival curve. The data demonstrate that lower doses of MA-EBOV (1 and 10 FFU) produce uniform lethality, whereas higher doses (>100 FFU) do not, resulting in nearly 30% survival in some cases. Notably, the difference between the survival curves from groups inoculated with 10 and 1000 FFU was significant ($p = 0.0230$, Log-rank test, GraphPad Prism 7). Animal studies were performed within the containment level 4 facilities at the Canadian Science Centre for Human and Animal Health, Public Health Agency of Canada, Winnipeg. All work was approved by the institutional animal care committee in accordance with guidelines from the Canadian Council on Animal Care. Animals were acclimatized for seven days prior to infection, and monitored daily, and were given food and water *ad libitum*.

2.2. Treatment

The antibody treatment regimens employed in conjunction with the mouse model are relatively straightforward. MA-EBOV-infected mice are typically treated a single time, on day 1 or 2 post-infection, with antibody that is delivered intraperitoneally (Table 3). The most commonly used dose of antibody is 100 μg per antibody per animal [12,13,24–27,33,34,42], which equates to approximately 5 mg/kg (assuming an average mouse weight of 20 g), although both higher (200 to 500 μg/animal) [28–32,35–38,40,42,43] and lower doses (2 to 50 μg/animal) [12,24,28,32,41,42] have been used (Supplementary Table 1). Doses lower than 100 μg/animal given later during infection increase the stringency of the evaluation, and this additional rigour, while not necessary, may be useful in identifying more potent antibodies for further pre-clinical development. In many studies, treatment has been delayed until day 2 post-infection or later [12,25,28–33,43]; however, treatment on day 1 post-infection represents the earliest time point at which the antibody should be expected to demonstrate efficacy (*i.e.*, significantly increase survival rates compared to control-treated animals). Likewise, multiple treatments in this particular model are uncommon, implying that a potential antibody therapeutic should be expected to show efficacy after only a single dose in order to move forward in development.

The remaining mouse models differ slightly from the MA-EBOV mouse model in the administration of treatment (Table 3 and Supplementary Table 1). The SUDV IFNAR$^{-/-}$ mouse model uses monoclonal antibody doses that range from 100 μg/animal to 500 μg/animal, with 200 and 500 μg used most frequently [26–32,34–36,43–46]. At least two doses of antibody are typically administered (intraperitoneally) with at least two days between treatments, although successful single-dose trials have been reported [26,28,31,32,34,43]. Conversely, the limited number of efficacy studies that have so far been performed with the marburgvirus mouse models restricts the ability to generalize a typical treatment regimen [37–39,49]. Thus far, multiple doses have been administered per animal at both high (500 μg/animal) and low (100 μg/animal) concentrations. In all published studies to date, the initial antibody treatment has been given either before or within an hour of the time of infection, with the last treatment as late as 4 days post-infection. Additional studies are therefore required in order to determine the best possible set of experimental conditions to use when performing antibody efficacy studies against the marburgviruses.

3. Guinea pigs

Outbred guinea pigs (strain Hartley) are frequently used to evaluate anti-filovirus countermeasures, and they are typically regarded as a more stringent model system with better predictive efficacy than the mouse models [7,60]. Guinea pigs better recapitulate filovirus disease as it is observed in humans and NHPs, and they offer several advantages, including ease-of-handling, low cost, and availability [18]. Moreover, guinea pigs are large enough to permit serial sampling, but still small enough to accommodate in most space-restricted high-containment laboratories. Like the immunocompetent mouse models, however, the guinea pig models all rely on the use of rodent-adapted filoviruses. To date, guinea pig-adapted (GPA) EBOV, SUDV, MARV, and RAVV have been described [18], and all have been used in efficacy evaluation of monoclonal antibody therapeutics (Table 2 and Supplementary Table 1). Unsurprisingly, the majority of these studies have focused on antibody therapeutics that, at a minimum, target EBOV, making the GPA-EBOV model the most commonly used [8,12,28,32,40,42,43,61–64]. However, since guinea pigs offer the only immunocompetent rodent model for SUDV, the GPA-SUDV model is also used frequently [26,28,32,43,65]. The limited reports of efficacy testing using the marburgviruses in the guinea pig model likely reflects the relative paucity of candidate antibodies targeting these viruses [40,66]. It is also worth noting that strain 13 guinea pigs, which are also susceptible to severe disease caused by GPA-EBOV [67], are rarely used in monoclonal antibody efficacy screening [64].

3.1. Inoculation & treatment

The majority of studies using the guinea pig model for antibody efficacy testing have used an intraperitoneal inoculation dose of 1000 LD_{50}, which seems to result in consistent uniform lethality, at least for GPA-EBOV and GPA-SUDV (Table 3 and Supplementary Table 1) [8,12,28,32,42,43,62,63]. Notably, several independently derived variants of GPA-EBOV exist, and different studies have made use of different variants [8,12,28,32,40,42,43,61–63]. The most commonly used variant for antibody efficacy testing appears to be GPA-EBOV/8mc (variant Mayinga), which is routinely used at a dose of 1000 LD_{50} (roughly 40 PFU in this case). Only a single variant of GPA-SUDV (variant Boniface) has been described to date [68], and it has been used at an inoculation dose of both 1000 PFU and 1000 LD_{50} [26,28,32,43,65], with uniform or near-uniform lethality. The few studies to have used GPA-MARV or -RAVV in antibody efficacy testing also used 1000 PFU and 1000 LD_{50} inoculation doses [40,66].

Regardless of the virus, guinea pigs are typically treated a single time, on day 1 to 4 post-infection, although treatment has been delayed up to day 7 post-infection (Table 3 and Supplementary Table 1). The most commonly used dose of antibody therapeutic is 5 mg per antibody per animal (roughly 15–20 mg/kg) [8,12,26,28,32,40,42,61–63,65]; however, doses as low as 2.5 mg/animal [42,62] and as high as 10 mg/animal [66] have also been used. Due to practical constraints imposed by work in high containment laboratories, all treatments are delivered intraperitoneally, despite the fact that this route may not be the most effective way to distribute antibody systemically.

4. Hamsters

Hamster models have been established for both EBOV and MARV, but their use in countermeasure development has so far been limited

Table 3
Commonly used infection and treatment regimens for monoclonal antibody evaluation.

Animal (strain)	Virus inoculation					Antibody treatment		
	Virus	Variant	Target dose	Route	Expected lethality	Dose per animal	Frequency	Route
Mouse (BALB/c)	MA-EBOV	Mayinga	10 PFU (~1000 LD_{50})	IP	~100%	100 μg total (~5 mg/kg)	Once (1 or 2 DPI)	IP
	MA-MARV	Ci67	1000 PFU	IP	~100–60%	100 μg total (~5 mg/kg)	Multiple (early)	IP
	MA-RAVV		1000 PFU	IP	~100–90%	500 μg total (~25 mg/kg)	Multiple (early)	IP
Mouse (IFNAR$^{-/-}$)	SUDV	Boniface	1000 PFU	IP	~100–70%	200–500 μg total (~10–25 mg/kg)	Twice (1 DPI & 3–5 DPI)	IP
Guinea Pig (Hartley)	GPA-EBOV	Mayinga	1000 LD_{50}	IP	~100%	5 mg total (~15–20 mg/kg)	Once (1–4 DPI)	IP
	GPA-SUDV	Boniface	1000 LD_{50}	IP	~100%	5 mg total (~15–20 mg/kg)	Once (1–4 DPI)	IP
	GPA-MARV	Angola	1000 PFU	IP	~100%	10 mg total (~15–20 mg/kg)	Once (1–4 DPI)	IP
	GPA-RAVV		1000 PFU	IP	~100%	10 mg total (~15–20 mg/kg)	Once (1–4 DPI)	IP
Ferret (Domestic)	EBOV	Makona	1000 $TCID_{50}$ or 1000 PFU	IM/IN	~100%	15–20 mg per Ab (~20–30 mg/kg)	Twice (3 & 6 DPI)	IP
	SUDV	Gulu	1000 $TCID_{50}$ or 1000 PFU	IM/IN	~100%	15–20 mg per Ab (~20–30 mg/kg)	Twice (3 & 6 DPI)	IP
	BDBV		1000 $TCID_{50}$ or 1000 PFU	IM/IN	~100%	15–20 mg per Ab (~20–30 mg/kg)	Twice (3 & 6 DPI)	IP
Rhesus Macaque	EBOV	Kikwit/Makona	1000 PFU	IM	~100%	50 mg/kg total	Thrice, at most (starting at 5 DPI)	IV
	SUDV	Boniface	1000 PFU	IM	<100%	50 mg/kg total	Thrice, at most (starting at 5 DPI)	IV
	BDBV		1000 PFU	IM	~60–75%	50 mg/kg total	Thrice, at most (starting at 5 DPI)	IV
	MARV	Angola	1000 PFU	IM	~100%	50 mg/kg total	Thrice, at most (starting at 5 DPI)	IV
	RAVV		1000 PFU	IM	~100%	50 mg/kg total	Thrice, at most (starting at 5 DPI)	IV

MA, mouse-adapted; GPA, guinea pig-adapted; LD50, 50% lethal dose; TCID50, 50% tissue culture infective dose; PFU, plaque forming units; IP, intraperitoneal; IN, intranasal; IM, intramuscular; IV, intravenous; DPI, days post-infection.

(Table 2 and Supplementary Table 1) [18]. While hamsters possess many of the advantages associated with other rodent models, they also offer a remarkably faithful recapitulation of filovirus disease. On the other hand, this model relies upon rodent-adapted viruses and it suffers from a relative lack of available research tools. Additionally, little data exist demonstrating the predictive efficacy of this relatively new model system, perhaps discouraging its widespread adoption. To date, only a single antibody efficacy evaluation has been performed using the hamster model [69]. Hamsters were intraperitoneally inoculated with 100 LD_{50} (~1 PFU) of HA-MARV and treated intraperitoneally with a single dose of 1 mg (~10 mg/kg) of antibody administered at 8 h or 1, 2, or 3 days post-infection. Whether the field continues to pursue this promising but under-used animal model remains to be seen.

5. Ferrets

Ferrets are a remarkable model system in that they are susceptible to wildtype—as opposed to host-adapted—EBOV, SUDV, and BDBV [50,51,70]. Disease caused by these viruses is uniformly lethal and highly similar to what is observed in humans, making the ferret a potentially valuable model for secondary efficacy screening, after rodent evaluation and prior to final evaluation in NHPs. This model is particularly useful for efficacy evaluation against BDBV, for which no other small animal model has been described [18]. Nevertheless, ferrets are not without their disadvantages. The animals are practically and logistically more difficult to handle within high containment laboratories, thereby increasing the complexity and cost of experiments; there are few ferret-specific research tools available to work with these animals; and there is very little known about their predictive efficacy [7]. Moreover, MARV and RAVV are non-pathogenic in ferrets [71,72], mitigating the value of this model for evaluating pan-filovirus countermeasures. So far, only a handful of studies have been published using ferrets for antibody efficacy testing, and the majority of these have used BDBV (Table 2 and Supplementary Table 1) [26,28,29,65].

5.1. Inoculation & treatment

The target inoculation dose for ferrets is 1000 $TCID_{50}$ or 1000 PFU, although back-titration has revealed that much smaller doses can also result in uniform lethality (Table 3 and Supplementary Table 1). Both the intranasal and intramuscular route can be used with the expectation of a similar disease course. In the few efficacy studies that have been reported, ferrets have been treated with 15–20 mg per antibody (approximately 20–30 mg/kg) delivered twice, usually on days 3 and 6 post-infection [26,28,29,65]. As is the case with the guinea pig model, antibody is delivered intraperitoneally, largely for practical reasons. It is also worth noting that since EBOV displays accelerated disease kinetics in the ferret model compared to BDBV and SUDV, earlier and/or increased treatment doses may be necessary in order to observe efficacy (X.Q., unpublished observations) [65].

6. Nonhuman primates

All promising anti-filovirus countermeasures are ultimately evaluated in either rhesus or cynomolgus macaques, which are considered the gold-standard filovirus model [21,73]. Both rhesus and cynomolgus macaques are susceptible to wildtype, human-pathogenic filoviruses, and they develop disease that closely parallels what is observed in humans. Moreover, these animals permit serial sampling, thus allowing for a thorough characterization of disease course, and they offer realistic inoculation and treatment routes. Although NHP experiments are costly and logistically challenging, they are the most stringent model system in which to complete pre-clinical evaluation for filovirus countermeasures, and they help satisfy the requirements for the FDA Animal Rule [74]. Owing to the slightly extended disease course in rhesus macaques compared to cynomolgus macaques, the former are typically chosen for countermeasure development, including antibody efficacy testing, although the latter are occasionally used [73]. As is the case with the small animal models, the majority of antibodies evaluated in the macaque models have targeted EBOV [8–11,17,61,62,65,75–77], though

studies involving SUDV [65], BDBV [65,78], MARV [66], and RAVV [66] have been reported (Table 2 and Supplementary Table 1).

6.1. Inoculation & treatment

The inoculation dose and route for the NHP model has been well established within the field for many years. Typically, 1000 PFU of virus is delivered intramuscularly, emulating a needle-stick exposure and creating a relatively high bar for any potential countermeasure to overcome [21,79]. Such an inoculation with EBOV produces uniform lethality, and the same is essentially true for MARV and RAVV, with variation depending on the virus variant and primate species [59]. SUDV infection occasionally results in survivors—again depending on the variant, primate species, and inoculation route used—while BDBV does not consistently produce uniform lethality [3,73], and this must be kept in mind when designing experiments.

With respect to antibody therapeutics, the most commonly used target dose is 50 mg/kg per antibody or antibody cocktail, delivered intravenously on multiple days post-infection (Table 3 and Supplementary Table 1). ZMapp, for instance, showed 100% efficacy when administered to EBOV-infected rhesus macaques at 50 mg/kg on days 5, 8 and 11 post-infection [8]. Following from this precedent, clinically useful antibody therapeutics should therefore be expected to show at least some efficacy even when treatment is delayed past 4 days post-infection. Indeed, recent studies have reported complete protection in animals infected with EBOV (variant Kikwit) and treated with 50 mg/kg or less, even after only one or two doses delivered beginning as late as day 5 post-infection [61,65]. Likewise, similar efficacy has been reported for the few antibody therapeutics that target SUDV, BDBV, MARV, and RAVV [65,66,78]. Ultimately, as antibody therapeutics continue to be refined and optimized, it is reasonable to expect treatments to be delivered less frequently at doses lower than 50 mg/kg; however, whether such austere regimens will prove effective or feasible in human cases remains to be seen.

7. Future perspectives

The development of monoclonal antibody therapeutics to treat filovirus disease, although progressing rapidly, is still in its infancy. While this therapeutic strategy offers tremendous potential, the field has primarily focused on antibody countermeasure development against EBOV, despite the fact that the remaining human-pathogenic filoviruses may also pose considerable risk to global public health and biosecurity. Not only must the field continue to develop monoclonal antibody therapeutics against the other filoviruses, but, ideally, it will work towards the goal of developing an antibody or cocktail of antibodies capable of treating all known filoviruses. Several strides towards this goal have already been made, but future work is still required.

The pre-clinical development of filovirus antibody therapeutics depends heavily on a variety of animal models required for efficacy evaluation. The mouse model is almost always the first system chosen to evaluate any experimental countermeasure against filoviruses, and monoclonal antibodies are no exception. However, immunocompetent mouse models do not exist for all filoviruses, namely SUDV and BDBV, so primary efficacy evaluation must also occasionally occur in guinea pigs and ferrets. These latter two animal models also serve as valuable systems for secondary screening, with guinea pigs in particular offering exceptional predictive efficacy. Whether hamsters will ever see widespread adoption as a suitable animal model in which to perform efficacy screening, at least against EBOV and MARV, remains to be seen. Ultimately, all potential therapeutics that, at a minimum, show efficacy in one of the small animal models must be evaluated in the gold-standard NHP model (cynomolgus or rhesus macaques), which is currently the only model in which all wildtype, human-pathogenic filoviruses show at least some degree of virulence. While thorough efficacy testing in numerous animal models is onerous, it is likely the easiest path towards eventual clinical licensure *via* the FDA Animal Rule. In effect, this rule states that when human clinical trials are not practical or ethical, efficacy testing can be performed in one or more immunocompetent animal models, so long as that model recapitulates human disease following infection with preferably wildtype virus using a realistic challenge dose and route [74]. Thus, the continued development of anti-filovirus monoclonal antibody therapeutics depends heavily on the use (and refinement) of multiple animal models.

The value of the animal models, in turn, depends not only on our understanding of filovirus disease in these systems, but also on the ways in which we design efficacy experiments using these models. Indeed, several important variables must be considered when evaluating a new monoclonal antibody, not to mention the choice of animal model in the first place. The virus inoculation dose and route can affect the interpretation of results, as is the case with MA-EBOV used at higher but not consistently lethal amounts in the BALB/c mouse model. Likewise, the antibody treatment doses, routes, and schedules of administration can all affect the outcome of evaluation, and the field must balance therapeutically realistic treatment regimens within the limitations imposed by the model. Our intentions with this review were to collate the variables associated with monoclonal antibody efficacy in filovirus animal models and present them in a meaningful way that will serve as a resource for the field. Notably, we understand the value of differing but well-controlled studies, and we are not necessarily advocating for the complete standardization of animal experiments within the field. We sought only to understand how the field is already performing antibody efficacy experiments, to compare and contrast the different methodologies in a meaningful way, and, in so doing, create a resource that will facilitate the continued development of countermeasures to treat filovirus disease.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2019.02.004.

Acknowledgements

This work was supported by the Public Health Agency of Canada.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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