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Marburg virus disease: A summary for clinicians

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ABSTRACT

Objectives: This article summarizes the countermeasures for Marburg virus disease, focusing on pathogenesis, clinical features and diagnostics. There is an emphasis on therapies and vaccines that have demonstrated, through their evaluation in nonhuman primates (NHPs) and/or in humans, potential for use in an emergency situation.
Methods: A standardized literature review was conducted on vaccines and treatments for Marburg virus disease, with a focus on human and nonhuman primate data published in the last five years. More detail on the methods that were used is summarized in a companion methods paper.
Results: The study identified six treatments and four vaccine platforms that have demonstrated, through their efficacy in NHPs, potential benefit for treating or preventing infection in humans.
Conclusion: Succinct summaries of Marburg countermeasures are provided to give the busy clinician a head start in reviewing the literature if faced with a patient with Marburg virus disease. Links to other authoritative sources of information are also provided.

Introduction

This is the first in a planned series on the management of highly hazardous communicable pathogens that may warrant specialized infection control measures and lack licensed countermeasures.

Marburg virus, a member of the filovirus family, has caused outbreaks in sub-Saharan Africa and can cause severe illness with high case fatality rates (Brauburger et al., 2012). Person-to-person spread may occur in household or nosocomial settings, where infection control modalities are sub-optimal. Although Marburg was discovered over 50 years ago, licensed prophylactic or therapeutic countermeasures have yet to be developed. Since the West African Ebola outbreak, increased effort has focused on Marburg in addition to Ebola (Olejnik et al., 2019).

As was the case with Ebola, it is anticipated that future outbreaks of Marburg virus disease (MVD) will trigger interest in the use of investigational products. The clinical features and risks of spread of MVD closely resemble Ebola virus disease (EVD); supportive care, infection control and other response measures (e.g. need for safe burials) are identical. However, much less is known about MVD than EVD because there has not been a recent large-scale outbreak of MVD or equivalent in scale to the EVD 2014–2016 West Africa outbreak. In addition, because the most promising specific therapies for EVD are monoclonal antibodies (Mabs), those that appear beneficial for EVD are likely inapplicable for MVD treatment. Similarly, vaccination platforms, such as the vesiculo stomatitis virus vaccine using antigens for EVD, have not demonstrated cross-protection for MVD (Jones et al., 2005).

Methods

This study summarized the recently published literature specific to MVD, in order to provide a practical list of potential countermeasures. These are summarized in the accompanying tables. The review involved a MeSH (National Center for Biotechnology Information, 2019) search string (customized for MVD) and divided the therapeutic evidence into categories: pre-exposure prophylaxis, post-exposure prophylaxis, treatment, infection prevention and control, and diagnostics. The literature review focused on the past five years; older data describing clinical features and incubation periods were included. Title, abstract and full text reviews of appropriate manuscripts, reviews and book

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Box 1. The pathogen

Marburg virus is an enveloped, non-segmented, single-stranded, negative-sense RNA virus in the filovirus family. There is a single species of Marburg – *Marburg Marburgvirus* – which includes two viruses with ~20% divergence: Marburg and Ravn virus. Marburg variants, with less genomic differences, include Marburg Musoke, Angola, an unnamed variant from the original 1967 outbreak (Ci67), and isolates within a variant (<7% divergence: Pop, Ci67). Marburg Angola, isolated from the largest outbreak, appears to be the most pathogenic and yields a more rapid disease course in NHPs. The Marburg glycoprotein (GP) is the only viral protein on the cell surface and has been the primary target for investigational viral vaccines.

Box 2. Epidemiology

Animal hosts: Presumed to be the Egyptian fruit bat (*Rousettus aegyptiacus*), based on epidemiologic linkage to outbreaks in caves or among miners in sub-Saharan Africa. RNA, antibodies and viral isolation by culture have been demonstrated in a small minority of bats. In addition, perpetuation of the virus in *R. aegyptiacus* has been demonstrated.
Transmission: Initial transmission to humans likely occurs from bats or another intermediate host (e.g. NHP, bush meat), but route and specific body fluid involved (saliva from bat, guano, urine) is unknown. Transmission to humans by direct contact with blood or body fluids of infected individuals occurs, with the majority of spread occurring through unprotected contact in the household or healthcare setting. There is a single reported case of live virus isolated from the aqueous humor of a human survivor >2 months after infection, so some degree of protected space persistence occurs, but there is less data on Marburg survivors than for Ebola. Although frank airborne transmission has not been demonstrated in human outbreaks, droplet spread to mucous membranes presumably occurs. Infection by direct application of aerosol to the airways has been demonstrated in animal models. Post-infection sexual transmission and recovery of virus in the semen have been demonstrated.
Human infections: The first outbreak occurred in 1967 in Germany and Yugoslavia related to importation of African Green monkeys (*Cercopithecus aethiops*) from Uganda. That outbreak resulted in 31 cases and seven deaths, some of which spread by household or nosocomial contact. Since then, there have been nearly 600 cases in outbreaks originating in the following countries: Uganda, Democratic Republic of Congo (DRC) and Angola. There have been fewer outbreaks of Marburg than Ebola. Marburg was presumed to be less deadly than Ebola, until two large outbreaks occurred with high fatality rates. The first, in 1998–2000 in the DRC (Durba), was associated with gold mining and resulted in 128 deaths and a case fatality rate of 83%, and was followed by the largest outbreak thus far, in Angola, in 2005, with 329 deaths and a case fatality rate of 88%. The most recent outbreak of three cases occurred in Uganda in 2018. Household members and healthcare workers are at particular risk. Overall, case fatality rates from the outbreaks have ranged from 23–88%, likely related to the extent of supportive care available.

chapters were then conducted. Bibliography scans were also completed on review articles and meta-analyses.

Clinical features

Incubation period

The incubation period is estimated to be 3–21 days (typically 5–10 days), likely related to infectious dose and route (Brauburger et al., 2012). The original Marburg outbreak described a range of 5–9 days among patients with well-defined exposure dates (Martini, 1971; Stille and Boehle, 1971). A 2011 review noted a range of 3–13 days for filoviral (Zaire Ebolavirus and Marburgvirus) infection based on definitive exposure dates (such as a known laboratory accident) (Kortepeter et al., 2011). A study focused on Marburg calculated an incubation period of 2–26 days (Pavlin, 2014).

Pathogenesis

Following exposure of mucosa or abraded skin, or through a needle-stick or other penetrating injury, the virus gains entry to the blood or lymphatic system and infects monocytes, macrophages and dendritic cells. Early replication occurs in these cells, which are likely responsible for further dissemination to hepatocytes, endothelial cells, fibroblasts, and epithelial cells (Rougeron et al., 2015). Filovirus binding to host cells has been associated with several attachment factors, including a glycoprotein (GP) on the viral surface that mediates binding and entry. The GP surface unit (GP1) binds to cellular receptors, and an internal fusion loop (GP2) inserts into the cell membrane (Hoffmann et al., 2017). Ebola and Marburg entry and the deposition of their replication machinery appear related to intra-vesicular cleavage of glycoprotein by host proteases, such as cathepsins, as well as to fusion of viral GP with the host protein Niemann-Pick (Cross et al., 2018). This process facilitates release of the viral core into the cell cytoplasm, where replication occurs. Due to its location on the cell surface and importance in binding and entry, the GP has been a key target for the development of both Ebola and Marburg vaccines and monoclonal antibody therapeutics.

Significant viral replication occurs in target organs such as the spleen, liver and secondary lymphoid organs. The virulence and high morbidity/mortality of the disease appears related to unchecked viral replication (related in part to inhibition of IFN-1 synthesis), as people with ultimately fatal infections generally exhibit high viral loads (Rougeron et al., 2015).

This replication is facilitated by the virus's ability to undercut the host immune response by exploiting intracellular and extracellular immune-mediated antiviral pathways (Cross et al., 2018). A focus of therapeutics discovery is thus related to finding small molecule antivirals that inhibit viral replication. Specific Marburg viral proteins can impair or neutralize the innate immune response.

Although lymphocytes are not directly infected by the virus, apoptosis of T-lymphocytes and natural killer cells causes massive depletion of lymphoid cells in the spleen, liver, lymph nodes, and thymus, and influences the inability to mount an adaptive immune response (Brauburger et al., 2012; Rougeron et al., 2015). Cell death is likely linked to interactions with infected antigen-presenting cells and soluble mediators.

Different aspects of the response feed upon each other. The uncontrolled viral replication, a consequence of dysregulation of the innate and adaptive immune responses, also leads to cytokine

storm and impaired humoral response, ultimately resulting in multiorgan failure and death. Through different pathways, immune dysfunction results in: (1) increased vascular permeability, influenced by TNF-alpha, NO and other vasoactive compounds; (2) tissue damage, mediated through MCP-1 and IL-8; and (3) disseminated intravascular coagulation influenced by abundant tissue factor expressed by macrophages. In contrast to fatal infections, the inflammatory response is early and moderate in non-fatal infections (Rougeron et al., 2015).

Clinical spectrum of infection

Asymptomatic cases of Marburg infection have not yet been documented. One study conducted serological assessment of 121 household contacts for unrecognized and asymptomatic infection. Two unrecognized cases were found, but both were symptomatic upon further questioning (Borchert et al., 2006). Most Marburg infections result in severe illness with prostration, bleeding manifestations and multiorgan failure.

Clinical course

Although there have been fewer outbreaks of Marburg than Ebola, and hence fewer descriptions of disease, some of the most detailed early clinical observations of filoviral hemorrhagic fever come from Marburg outbreaks, including the 1967 outbreak in Marburg and Frankfurt, Germany, and Belgrade, Yugoslavia, as well as a subsequent outbreak among three travelers cared for in South Africa (Martini, 1971; Gear et al., 1975).

Following the incubation period, patients usually become abruptly ill with non-specific symptoms such as fever, chills, headache, odynophagia, myalgia, vomiting, and diarrhea. Early cases may be missed, owing to similarities with more common infections such as malaria, typhoid, or rickettsial illness. Rash is a common feature early in MVD, and is described as non-pruritic, erythematous and maculopapular. It may begin focally, then become diffuse and confluent. As noted during the original outbreak, "It began between the fifth and seventh day at the buttocks, trunk, and outside of both upper arms as a distinctly marked, pin-sized red papula around the hair roots," which lasted up to 24 h, then developed into a maculopapular rash, which later coalesced (Martini, 1971). Conjunctival injection may also occur early.

During MVD, large swings in body temperature have been noted, encompassing hyper- and hypo-pyrexia. In the original outbreak, tachycardia corresponding to temperature elevation was only seen in fatal cases. Laboratory abnormalities include leukopenia and lymphopenia, hypokalemia, normal to elevated levels of amylase, thrombocytopenia, and elevated liver enzymes. As illness progresses, elevations in prothrombin time and partial thromboplastin time, as well as clinical bleeding, may occur. Patients may develop multiple foci of mucosal hemorrhage, typically in the conjunctivae, along with easy bruising or persistent bleeding from venipuncture sites. Renal function may be initially normal, although renal function is often impaired and dialysis may be required by the end of the first week of illness. Severe cases progress from prostration and obtundation to hypotension, shock and multiorgan failure. In the West African outbreak of EVD, significant gastrointestinal disease was described, with vomiting and diarrhea leading to volume loss, acid base disturbances and electrolyte imbalances (Duraffour et al., 2017). Similar features can occur with Marburg and a recent review summarized the clinical features of MVD (Bauer et al., 2019).

Mortality risk factors

The case fatality ratio has ranged from 23–90% (CDC, 2019). Most fatal cases succumb during the second week of illness, a mean of 9 days after onset. Those who survive this period are likely to recover. Risk factors for severe disease or death have been reported for Ebola, but not Marburg. These include old or very young age, as well as higher viremia levels and elevated levels of AST, BUN, creatinine, certain cytokines (IL6, IL8, IL10, macrophage inflammatory protein 1β), ferritin, and D-dimer, decreased albumin and calcium, and lack of antibody response (Hutchinson and Rollin, 2007; Rollin et al., 2007; Schieffelin et al., 2014; WHO Ebola Response Team et al., 2015).

Pathology

Autopsies have demonstrated focal necrosis without inflammation in the liver, spleen, testes, ovaries, and the pancreas, and signs of hemorrhagic diatheses in all organs. Glial nodule encephalitis has been noted throughout the brain. Significant renal damage and signs of tubular insufficiency also occur. Lymphatic tissue demonstrates plasmacellular and monocytoidal transformation. Basophilic bodies have been noticed near necrotic cells or as inclusion bodies in parenchymal cells (Martini, 1973).

Sequelae

Survivors have experienced prolonged convalescence and numerous sequelae, including myalgia, exhaustion, hyperhidrosis, skin desquamation, amnesia, testicular atrophy, decreased libido, and hair loss (Brauburger et al., 2012; Martini, 1973). Live virus has been recovered from samples of semen and aqueous humor for up to 3 months after illness, and Marburg virus has also been found to persist in the testes of nonhuman primates that have survived (Gear et al., 1975; Kuming and Kokoris, 1977; Coffin et al., 2018). One survivor transmitted infection to his wife through sexual intercourse >2 months after illness (Martini, 1973).

Diagnostic testing

Diagnosis of MVD can be made using multiple modalities, including culture, RT-PCR, serology, and immunohistochemistry, depending on the time course of the infection. Typical diagnostic samples include blood, other body fluids and tissue obtained at autopsy. Reagents for Marburg testing may not be as widely available as for Ebola. Clinicians in the United States (U.S.) should first contact their state health department regarding a patient/patient under investigation with suspected Marburg prior to submitting any specimens. If the state health department prefers that specimens go directly to the Centers for Disease Control and Prevention (CDC) for testing, the specimens will be shipped to the Division of High Consequence Pathogens and Pathology (DHCPP), CDC (https://www.cdc.gov/ncezid/dhcpp/index.html [cdc.gov]) within the Viral Special Pathogens Branch (https://www.cdc.gov/ncezid/dhcpp/vspb/index.html [cdc.gov]). Other potential sites for such shipment include biosafety level 4 laboratories or the Diagnostics Systems Division at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) – 1-800-USA-RIID).

Potential treatment or prophylaxis countermeasures

Pre-exposure prophylaxis

No Marburg vaccines are approved in the U.S. or worldwide. There is no cross protection between Ebola and Marburg virus vaccines, although several constructs tested in cynomolgus macaques have demonstrated protection against both Marburg and Ravn viruses (Table 1). Three candidate Marburg vaccines (cAd3, MVA-BN-Filo and MARV DNA) are in Phase I clinical trials and one (MVA-BN-Filo) is scheduled for a Phase 2/3 clinical trial.

Table 1
Vaccines.

Vaccine	Manufacturer or source/contact	Description	NHP studies	Human use (INDs, case reports, phase 1 or 2)	Phase 3/RCTs	Regulatory approvals	Notes/special populations
cAd3		Chimpanzee adenovirus serotype 3 vector, encoding wild type (WT) glycoprotein (GP) from Marburg virus	No data for this construct for Marburg. Protection with other constructs: Ad26 alone (75%) better than Ad35 with Ebola. Ad26 plus Ad35 boost 100%. cAd3 prime followed by MVA boost was protective against Ebola	Phase 1 clinical trial with Marburg construct active, not yet recruiting (NCT03475056)			
MVA-BN-Filo	Janssen Pharmaceuticals, Titusville, NJ (of Johnson and Johnson)	Modified vaccinia Ankara vector, encoding glycoproteins from Ebola, Sudan, and Marburg viruses, and Tai Forest virus nucleoprotein	No data for this construct for Marburg. An Ebola vaccine demonstrated protection out to 10 months in Ebola-infected NHPs using a cAd3 prime followed by MVA boost 8 weeks later	Phase 1 trial for MVA-BN-filo in prime-boost with Ad26.ZEBOV. Better immune response after Ad26.ZEBOV primary. Sustained Ebola GP immunity after either primary followed by alternate boost. Response to Marburg antigens not measured	Phase 2/3 trials planned. Use of construct against Ebola planned in response to DRC Ebola outbreak 2019		
MARV DNA plasmid vaccine		Marburg DNA plasmid expressing GP from Marburg Angola	Study using a DNA prime/boost vaccine demonstrated protection, but all animals developed signs/symptoms	90% antibody response in Phase 1 trial, 10 people; 1 discontinued for non-life-threatening side effects; 4th dose at 12 wks improved waning antibody titers			
rVSV-MARV-GP		Recombinant vesiculo stomatitis virus vector for Marburg GP	Several tried with good immune response. Sustained IgG response and protection against clinical illness: protected 20–30 min (5/5), 24 h (4/6) and 48 h (2/6) post-challenge	No human trials, although a similar Ebola vaccine has now been used in three different Ebola virus outbreaks in Africa is now licensed			
VLP		Virus-like particles with GP	Vaccine against Musoke, Ci67, Ravn with Ab response to all three strains and cross-protection after challenge 4 weeks later				

Multiple Marburg candidate platforms (rVSV, VLP, Adenovirus, DNA) have demonstrated protection in NHPs (Reynolds and Marzi, 2017).

Adenovirus vectored vaccines

Several adenovirus-based vaccines have been studied for EBOV, but studies are limited for MARV. Recombinant adenovirus serotype 5 (rAd5) is the most commonly used vector for glycoprotein (GP) vaccines. In one study, macaques were given a single dose of rAd5 vaccine expressing MARV-Angola GP. They were challenged with homologous MARV 4 weeks later and none developed clinical illness. A similar response was seen in four macaques receiving three doses of MARV-Angola GP DNA prior to vaccine in a prime-boost strategy (Geisbert et al., 2010a).

The complex adenovirus (CAdVax) platform uses five antigens: EBOV, SUDV and MARV (Ravn, Musoke, and Ci67) glycoproteins, and EBOV and MARV-Musoke nucleoproteins. Cynomolgus macaques were given this vaccine using a prime-boost strategy, and challenged with EBOV, SUDV and MARV-Musoke/Ci67. Antibodies were produced against all five filoviruses and no animals developed clinical illness (Swenson et al., 2008a).

Use of adenovirus-based vectors has been limited by pre-existing immunity in the population. To address this, less common serotypes have been employed, and oral or nasal vaccine administration has been used in animal models. Ad26 and Ad35-vectored vaccines using Ebola GP demonstrated less protection than Ad5, but Ad26 protected three of four NHPs after Ebola challenge, and provided protection in a prime-boost strategy employing an Ad35 vectored Ebola vaccine (Geisbert et al., 2011). Similarly, chimpanzee Adenovirus 3 has been assessed as a potential vector for GP. Four animals in two groups of different doses survived post-vaccination challenge with Ebola virus (Stanley et al., 2014) but durable protection was not achieved. Use of a cAd3 prime followed by a Modified Vaccinia Ankara boost at 8 weeks, with challenge 10 months later, demonstrated 100% protection against Ebola. Similar studies in NHPs have not been performed with Marburg, but clinical trials with similar vaccines have been conducted. A Phase 1 clinical trial of a cAd3 Marburg vaccine is currently ongoing (NIH 2020).

A Phase 1 clinical trial of Ad26.ZEBOV and MVA-BN-Filo vaccines, published in 2016, included 87 participants who were randomized to receive Ad26.ZEBOV or MVA-BN-Filo (modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola, Sudan, Marburg, and Tai Forest viruses). After primary immunization, subjects were boosted with the alternate vaccine at 14, 28 or 56 days. There were no vaccine-related serious adverse events. 97% of Ad26.ZEBOV recipients and 23% of MVA-BN-Filo recipients had detectable IgG response 28 days after primary immunization, and all recipients had detectable IgG levels at 21 days and 8 months after receiving the alternate vaccine boost. Phase 2/3 trials of both vaccines are planned (Milligan et al., 2016).

DNA vaccines

DNA vaccines against filoviruses have good safety profiles in NHP trials, are easy to produce, and have the potential to induce humoral and cellular immunity; however, these have demonstrated limited immunogenicity in clinical trials (Lu et al., 2008; Falzarano et al., 2011; Martin et al., 2006). DNA vaccines containing MARV-Musoke GP and MARV-Angola GP tested in cynomolgus macaques produced an IgG response and protection from homologous challenge;

however, all developed clinical illness, suggesting that the IgG response alone did not control infection (Geisbert et al., 2010a; Riemenschneider et al., 2003). DNA-based vaccines have been used with greater success as part of a prime-boost strategy, such as with an adenovirus vector. A Marburg DNA plasmid vaccine (VRC-MARDNA025-00-VP) expressing MARV Angola DNA has completed Phase 1 clinical testing. Ten people received vaccine (0, 4, 8 weeks): 90% had antibody responses; seven received a fourth dose at 12 weeks, which boosted waning antibody titers. No phase 2/3 trials are currently underway.

Recombinant vesicular stomatitis virus (rVSV) vaccine

Vesicular stomatitis virus (VSV) is a negative-strand RNA virus of the Rhabdoviridae family. MARV rVSV vaccines are replication-competent, and contain MARV GP in place of its innate surface membrane glycoprotein; several have been studied in NHPs (Geisbert et al., 2008). A vaccine using three VSV vectors containing MARV, EBOV and SUDV GP given to NHPs produced antibody responses to all three components, with 100% cross-protection against MARV, EBOV, SUDV, and TAFV 28 days after vaccination. One animal developed detectable viremia (Geisbert et al., 2009). Another study demonstrated sustained IgG response and protection against clinical illness in cynomolgus macaques challenged with MARV 14 months after rVSV-MARV-GP vaccination (Mire et al., 2014).

During the 2014–2016 West African EBOV epidemic, an rVSV-EBOV vaccine was successfully used in a ring vaccination trial (Henao-Restrepo et al., 2017). It has since been employed during two recent EBOV outbreaks in the Democratic Republic of the Congo (DRC). The vaccine is now approved in both the U.S. and Europe. The rVSV platform appears promising for MARV; however, there are no Phase I clinical trials yet in progress.

Virus-like particles (VLP)

Marburg VLP (mVLP) vaccines have been produced using matrix protein VP40 and Marburg GP, producing VLPs similar in morphology to Marburg virions (Warfield and Aman, 2011). A VLP vaccine against MARV-Musoke, Ci67 and Ravn isolates was tested in NHPs and produced antibody responses to all three strains. Moreover, all demonstrated cross-protection when challenged with MARV 4 weeks later (Swenson et al., 2008b).

Post-exposure prophylaxis

Post-exposure prophylaxis (PEP) using a VSV-vectored vaccine that incorporates Marburg glycoprotein reduced deaths when given within 20–30 min (five of five protected), (Daddario-DiCaprio et al., 2006) 24 h (five of six protected), or as late as 48 h (two of six protected) after challenge with Marburg Musoke in rhesus macaques (Geisbert et al., 2010b). Post-exposure protection was afforded, depending on dose (high ~1000 pfu, low ~50 pfu), when rhesus macaques were vaccinated against the more virulent Marburg Angola variant within 20–30 min of challenge (Woolsey et al., 2018).

Treatment

Multiple pharmaceuticals active against Marburg are in development, including immunotherapeutics, phosphorodiamidate morpholino oligomers (PMOs), lipid-encapsulated small interfering RNAs, small molecule inhibitors, interferons, and antiviral nucleoside analogs, shown in alphabetical order (Table 2).

Galidesivir – BCX4430 (Biocryst Pharmaceuticals)

Galidesivir is a synthetic nucleoside analogue that inhibits viral RNA polymerase by acting as a non-obligate RNA chain terminator. Galidesivir has activity against numerous viruses (Togaviruses, Bunyaviruses, Arenaviruses, Paramyxoviruses, Coronaviruses, Orthomyxoviruses, Picrornaviruses, and flaviviruses). Groups of six cynomolgus macaques (Warren et al., 2014) were challenged with Marburg virus and then given twice daily IM injections of Galidesivir (15 mg/kg) 1, 24 and 48 h after challenge. All controls died. Five of six animals survived in the 1-h group; all survived in both the 24-h and 48-h groups. No overt signs of toxicity were noted. Lower viremia levels, decreased clotting times and improved liver enzyme levels were noted in treated animals. Galidesivir has also demonstrated post-exposure protection against Ebola virus in rhesus macaques.

Testing in humans

A phase 1 safety study was concluded in 2016, but results have not been published.

Favipiravir – T-705 (Toyama Chemical Co., Ltd)

Favipiravir, a synthetic guanidine nucleoside analog with broad-spectrum activity against multiple families of RNA viruses, is licensed in Japan for the treatment of influenza. Early work demonstrating efficacy in mouse models against Ebola virus led to interest in its use during the West African outbreak. The results of a large-scale trial (JIKI) in Guinea were inconclusive, although it appeared to have efficacy in patients with lower viremia levels (Ct value $\geq$20) (Sissoko et al., 2016). This trial used historical rather than concurrent controls. Another recently published study from Guinea demonstrated a trend to improved survival in the treated cohort, albeit without a significant survival benefit (Kerber et al., 2019). Bixler et al. demonstrated survival of five of six cynomolgus macaques challenged with 1000 PFUs (Marburg Angola) when favipiravir was given intravenously twice daily for 14 days, beginning on the day of challenge; oral dosing did not produce benefit (Bixler et al., 2018).

Remdesivir (Gilead Sciences)

Remdesivir is a prodrug of an adenosine analog with in vitro activity against Marburg. It has been successfully used to treat EVD in NHPs, and has recently demonstrated effectiveness in treating Marburg-infected cynomolgus macaques 4–5 days post-exposure at once daily doses of either 5 mg or 10 mg for 12 days (two doses, 50% and 83% survival, respectively) (Porter et al., 2020). Remdesivir was given to a nurse who had recovered from EVD, but developed meningoencephalitis 9 months later (Jacobs et al., 2016). Ebola was detected in blood at a lower concentration than in CSF, and was undetectable after 14 days of treatment, which included high-dose steroids. Remdesivir was also given to a premature infant born to a woman infected with Ebola during pregnancy. The infant also received leukocytes and ZMapp, tolerated the treatment well and was discharged from hospital (Dornemann et al., 2017).

Remdesivir was included in a four-drug randomized controlled therapeutic trial in the DRC (the PALM study) (Mulangu et al., 2019). The survival was lower in the remdesivir arm when compared with two monoclonal antibody preparations (REGN-EB3 and Mab114) and it was deprioritized for further use for Ebola. Widespread use of remdesivir has occurred as a countermeasure during the 2019–2020 COVID-19 outbreak under emergency use authorization, and there are numerous ongoing clinical trials with it (Beigel et al., 2020).

Interferon-beta

EVD in humans is associated with robust interferon alpha response, but little interferon beta (IFN-β) production. This finding has led to studies wherein IFN-β was administered after Marburg infection in macaques (Smith et al., 2013). Early treatment was associated with increased mean survival time, but did not alter mortality. The authors concluded that interferon beta might serve as an adjunctive therapy.

Table 2
Marburg countermeasures – treatment with antivirals.

Therapy	Manufacturer or source/contact	Description	NHP studies	Human use (INDs, case reports, phase 1 or 2)	Phase 3/ RCTs	Regulatory approvals	Notes/special populations
Antivirals							
NP-718-LNP	Tekmira/Arbutus Biopharma, Vancouver, BC, Canada	Small-interfering RNA targets nucleoprotein	100% survival (16 NHPs) with treatment 30 min–2 h post infection	Multiple microRNAs tested in humans, but not this product or any others for filoviruses			
BCX4430 (Galidesivir)	Biocryst Pharmaceuticals, Durham, NC	Synthetic nucleoside analogue that inhibits viral RNA polymerase	17/18 survive with treatment 1–48 h post infection. Also shown protection for Ebola	Phase 1 study completed 2016. Results not published			
AVI-7288 alone or in combination with AVI-7287 as AVI-6003	Sarepta Therapeutics, Cambridge, MA	Phosphorodiamidate morpholino oligomers with positive charges - AVI 7288/7287 Target NP/VP24 gene, respectively	83–100% protection with treatment 24–96 h post infection	With AVI-6003, no significant safety signals in two RCTs with 70 subjects			
Favipiravir (T-705)	Toyama Chemical Company, Ltd, Japan	Synthetic guanidine nucleoside analog with broad-spectrum antiviral activity against multiple families of RNA viruses	5/6 survived when begun IV on day of challenge, but not with oral doses	Inconclusive results in West Africa (JIKI) Ebola trial using historical controls. Lower viremia (Ct $\geq$20) fared better		Licensed in Japan for influenza. Has had broad human use	
GS-5734 (remdesivir)	Gilead Sciences, Foster City, CA	Monophosphoramidate prodrug of an adenosine analog with broad antiviral activity. Inhibits Marburg in vitro	Protected 50 and 83% of MARV-infected NHPs against lethal disease when initiated up to 4–5 days post-infection with Marburg	Female nurse recovered after treated for Ebola meningoencephalitis relapse. Multiple human trials ongoing for COVID-19. Used for MEURI compassionate use in 2018–2019 DRC outbreak. Lower survival than antibody-derived products in PALM RCT for 2018/19 Ebola outbreak in DRC			35–36-week-old infant treated whose mother was infected with Ebola during pregnancy

Polyclonal concentrated IgG

Concentrated (polyclonal) IgG was derived from vaccinated NHPs that had survived challenge with Marburg (Table 3). An initial study gave three doses (100 mg/kg) to rhesus macaques following Marburg challenge, and resulted in 100% protection without viremia or observed clinical illness, but the animals did develop an IGM response. Re-challenge with Marburg 77 days later demonstrated complete protection. In a second study the first dose was given IV 48 h post-challenge, followed by doses on Day 4 and Day 8. All three animals survived, although one developed mild illness (Dye et al., 2012).

Monoclonal antibodies

After demonstrating protection against Marburg virus lethal challenge in mice, a panel of MAbs was studied in guinea pigs and NHPs. MR 191-N, a human monoclonal, was tested in rhesus macaques with 50 mg/kg IV given at Day 4 and Day 7 post-infection (Mire et al., 2017). All three treated animals survived. In a second study, four of five and five of five animals survived challenge with Marburg and Ravn viruses, respectively. The one treated non-survivor demonstrated the highest viremia level ($>10^7$ pfu/mL), but had an initial drop in viremia followed by rebound unrelated to generation of an escape mutant. The treated animals demonstrated illness and laboratory abnormalities, but these resolved after treatment, making this a potential candidate for therapy as well as prophylaxis.

Testing in humans

MR 191-N was used following a recent lab exposure, but details have not been published; human trials are being planned. Numerous licensed monoclonal therapies have been used for other diseases. A three-antibody cocktail (ZMapp) was tested in a randomized controlled trial (RCT) in humans with EVD during the West Africa outbreak and led to improved outcomes among recipients, compared with those receiving only supportive care. Since the outbreak concluded before sufficient numbers of patients were enrolled in the trial, statistical significance was not achieved. ZMapp was also tested as part of a four-drug RCT (the PALM study) during the 2018–2019 Ebola outbreak in the DRC (Mulangu et al., 2019). Alternative Mab preparations (mAb114 and REGN-EB3) have proven superior in reducing mortality. Given that the mAb1114 product consists of a single monoclonal antibody, whereas ZMapp and REGN-EB3 are cocktails of three monoclonals, it provides some credence that a single monoclonal preparation may be enough to treat other VHF illnesses such as MVD. Given their demonstrated efficacy against EVD, it is reasonable to consider Mabs as potential therapeutics against MVD.

Phosphorodiamidate morpholino oligomers (PMOs) with positive charges

PMOs inhibit mRNA translation through steric hindrance (Cross et al., 2018). The morpholino group is similar to a ribose base in RNA, and a methylene phosphorodiamidate linking moiety that physically binds to mRNA prevents translational machinery from accessing it. Addition of a piperazine residue provides a positive charge (PMO plus), believed to enhance binding to negatively charged mRNA and subvert development of resistant mutations. Once antisense PMOs bind to target mRNA they are highly stable and soluble, allowing high levels of inhibition and predictably low levels of toxicity.

Initial testing of AVI-6003 (combination of AVI-7287 and AVI-7288 that target MARV VP24 and NP, respectively) demonstrated

Table 3
Marburg countermeasures – treatment with antibodies.

Therapy	Manufacturer or source/contact	Description	NHP studies	Human use (INDs, case reports, phase 1 or 2)	Phase 3/ RCTs	Regulatory approvals	Notes/special populations
Polyclonal concentrated IgG		Concentrated IgG derived from previously vaccinated NHP survivors from Marburg challenge	100% protection (6 NHPs) with three doses starting 15–30 min or 48 h after infection				
Monoclonal antibodies							
MR 191-N	Mapp Biopharmaceuticals, San Diego, CA, and Kentucky Bioprocessing	Human monoclonal antibody made in Nicotiana tobacco plants binds the receptor binding site of Marburg GP	12/13 and 3/3 NHPs survived with treatment at D4/D7 or D5/D8 post infection	Used as an emergency IND for a U.S. lab exposure. Details not public			

a high level of protection against Marburg virus infection in mice, guinea pigs and cynomolgus macaques. In NHPs, survival was dose-dependent, with 100% protection demonstrated at doses of 20 mg/kg and 30 mg/kg when given 30–60 min post-exposure. However, a subsequent study demonstrated failure of the AVI-7287 component to protect NHPs. AVI-7288 appeared to be the active compound and was selected for further testing in cynomolgus macaques challenged with 1000 PFUs of Marburg (Iversen et al., 2012). Animals were given doses of 15 mg/kg per day for 14 days, starting at 24, 48 and 96 h post challenge, yielding 83%, 100% and 83% protection, respectively. Human dosing was extrapolated from this study (Heald et al., 2015; Warren et al., 2016).

Testing in humans

In an RCT, AVI-6003 was tested in humans at ascending doses (0.05–4.5 mg/kg) (Heald et al., 2014). It was well tolerated in 30 subjects, the most common adverse effects being gastrointestinal symptoms, headaches and dizziness. Grade 1 elevations of ALT and AST were noted in two subjects; mild elevations in amylase were seen in eight. In a subsequent RCT involving 40 healthy volunteers, subjects received daily infusions at doses ranging from 1–16 mg/kg (Heald et al., 2015). No safety concerns or serious adverse events were identified, although 10 participants developed headache or other mild side effects. The protective dose for humans, extrapolated from NHPs and AUC_{24}, is 9.6 mg/kg. Monte Carlo simulations supported a dose of 11 mg/kg to match mean protective exposure in NHPs.

Small interfering RNAs (siRNAs). SiRNAs interfere with the translation of mRNA by sterically blocking mRNA or by triggering cleavage of the DNA/RNA duplex. An initial study identified a siRNA – NP-718m – that targeted Marburg nucleoprotein. When encapsulated in lipid nanoparticles (ensuring cell entry by preferentially fusing with the endosomal membrane), this compound inhibited replication of Marburg in vitro and demonstrated broad protection against three Marburg strains in guinea pigs (Ursic-Bedoya et al., 2014). Further study of NP-718-LNP was undertaken in Marburg-infected rhesus macaques (Thi et al., 2014). Twenty-one animals were challenged with Marburg Angola (doses ranging 1000–1775 pfus) and received treatment (seven daily IV doses) with NP-718-LNP at 30–45 min, 24, 48, and 72 h post infection. All 16 treated animals survived. Clinical illness was much less severe and viremia levels lower in treated animals.

Testing in humans. At least 14 siRNAs have entered into clinical trials (www.clinicaltrials.gov) in which ~1500 healthy volunteers have been enrolled. However, none of those trials was designed for testing potential filovirus countermeasures. One of the main challenges has been to develop efficient systems to deliver accurate doses to targeted cells.

Infection prevention and control recommendations

Marburg patients might be optimally managed in specialized biocontainment units. In the absence of such, infection prevention and control guidelines for Marburg are similar to those for other viral hemorrhagic fevers, primarily consisting of barrier nursing techniques, including the use of personal protective equipment (PPE) such as gowns, gloves, masks, and face shields or goggles to prevent contact with blood or body fluids. Strict adherence to the correct use of PPE, with attention to hand hygiene and prevention of self-contamination, especially during doffing, is required. Patients should be placed in a single room with dedicated bathroom or commode. A U.S. Environmental Protection Agency-registered hospital disinfectant with efficacy against enveloped viruses should be used to disinfect environmental surfaces. Single-use medical equipment should be used when possible. Reusable equipment must be cleaned and disinfected according to the Spaulding Classification scheme (CDC, 2008). The CDC and World Health Organization have developed a manual of *Infection Control for Viral Haemorrhagic Fevers in the African Health Care Setting* (https://www.cdc.gov/vhf/abroad/vhf-manual.html). The CDC has also developed guidelines for managing Ebola patients in resourced settings (https://www.cdc.gov/vhf/ebola/clinicians/index.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fvhf%2Fabroad%2Fvhf-manual.html); these are also indicated for Marburg, and include focus on the use of PPE, waste management, cleaning and disinfection, and other aspects of management. Additional resources are available at www.netec.org.

To reduce the likelihood of occupational exposures, the use of needles and sharps should be minimized. Facilities should develop plans to manage employees who may have an exposure to blood or body fluids. Aerosol-generating procedures should be undertaken with extreme caution, preferably in an airborne infection isolation room, with providers wearing appropriate PPE, including respiratory protection. In the U.S., waste generated in the care of patients under investigation or patients with confirmed EVD is subject to procedures set forth by local, state and federal regulations. Extensive guidance is provided by the CDC on all aspects of care of patients with VHF, including guidance on hemodialysis, pregnant women, handling human remains, neonatal care, selection and use of PPE, cleaning and disinfection, and management of waste.

Summary and recommendations

The model for use of investigational countermeasures during outbreaks of EVD has been established, and a similar approach

would likely be taken during an MVD outbreak. Few of the countermeasures listed here have been tested in humans with MVD, and must be approached with caution through establishment of an FDA-approved investigational new drug (IND or emergency IND) protocol with informed consent. Sources of Marburg convalescent plasma are extremely limited, and the use of convalescent plasma did not appear beneficial during the 2014–2016 EVD outbreak in West Africa. Monoclonal antibodies against EVD appear more promising and, as noted above, an RCT testing four potential therapeutics was stopped early by the data safety monitoring board because of the apparent superiority of two products (Mulangu et al., 2019). MR-191 appears promising for Marburg, although it was used in a single human laboratory exposure and details surrounding that use are unavailable.

Although NHP data for favipiravir and remdesivir appear favorable, there are no data to indicate whether either would be beneficial in humans. Remdesivir, used in the PALM trial in the 2018–2019 Ebola outbreak in DRC for expanded access and an RCT, did not appear as beneficial as Mab preparations. It is unknown whether the same would apply to Marburg, but given that background it is reasonable to consider Mabs as potential first choices for treatment, if available. The siRNA and PMO products – NP-718-LNP and AVI-7288 – demonstrate good protection of NHPs against Marburg, and both have been tested in humans without any significant safety problems. However, neither has been shown to provide superior protection in humans, and given the lack of efficacy of those platforms for Ebola in humans, those products would likely not be first choice for use. Galidesivir demonstrates prophylactic efficacy against Marburg out to 24 h; phase 1 results are pending. Given the less robust response to EVD for the antiviral remdesivir, the antiviral Galidesivir might be a potential second choice below a monoclonal antibody preparation, such as MR-191-N.

Several vaccine platforms appear promising. Adenovirus constructs show promise using strains having limited circulation in humans, such as Ad26/35 or cAd3, but these have not been tested against Marburg. DNA vaccines appear less protective in NHPs and would likely need to be used in a prime-boost fashion and the lack of existing licensed platforms lower their priority for emergency use, from the authors' opinion. The recombinant VSV vaccine against Ebola is now licensed and has demonstrated safety and a reported efficacy of 97.5%. A similar platform appears to protect NHPs against Marburg. Although it has not yet been tested in humans, such an approach might hold similar promise if used for Marburg. VLPs also appear promising in NHPs but, again, have not been tested in humans, and there are no licensed VLP vaccines. Considering all these aspects, an adenovirus or VSV-vectored vaccine appear the most promising at this time.

Many lessons learned from the Ebola experience in Africa might be applied to future Marburg outbreaks. Notably, even if promising investigational therapeutics are used, supportive care – including close monitoring of vital signs, fluid resuscitation, electrolyte and acid base monitoring – are critical components of care that must be aggressively managed in order to optimize patient outcomes in any field trials. Fischer et al. (2019) Because medical countermeasures against Marburg are rapidly evolving, updated information will be provided at www.netec.org as it becomes available.

Conflict of interest

Dr. Kortepeter is a shareholder and Chair of the Scientific Advisory Board of Integrum Scientifics. No other potential author conflicts of interest noted.

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The content and views expressed in this manuscript are the responsibility of the authors and do not necessarily represent the official views of the Department of Health and Human Services Office of the Assistant Secretary for Preparedness and Response, nor are they intended to represent the views of the authors' individual institutions.

Ethical approval

The work described herein was solely a review of the literature and as such did not have a requirement for Institutional Review Board or Animal Use Committee approvals.

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