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REVIEW

Targeting Ebola virus replication through pharmaceutical intervention

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ABSTRACT

Introduction. The consistent emergence/reemergence of filoviruses into a world that previously lacked an approved pharmaceutical intervention parallels an experience repeatedly played-out for most other emerging pathogenic zoonotic viruses. Investment to preemptively develop effective and low-cost prophylactic and therapeutic interventions against viruses that have high potential for emergence and societal impact should be a priority.

Areas covered. Candidate drugs can be characterized into those that interfere with cellular processes required for Ebola virus (EBOV) replication (host-directed), and those that directly target virally encoded functions (direct-acting). We discuss strategies to identify pharmaceutical interventions for EBOV infections. PubMed/Web of Science databases were searched to establish a detailed catalog of these interventions.

Expert opinion. Many drug candidates show promising *in vitro* inhibitory activity, but experience with EBOV shows the general lack of translation to *in vivo* efficacy for host-directed repurposed drugs. Better translation is seen for direct-acting antivirals, in particular monoclonal antibodies. The FDA-approved monoclonal antibody treatment, Inmazeb™ is a success story that could be improved in terms of impact on EBOV-associated disease and mortality, possibly by combination with other direct-acting agents targeting distinct aspects of the viral replication cycle. Costs need to be addressed given EBOV emergence primarily in under-resourced countries.

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1. Introduction

Ebolaviruses are non-segmented, single-stranded negative sense RNA viruses that together with marburgviruses represent the archetypal members of the only two known genera of the *Filoviridae* family that cause disease in humans[1]. Members of the remaining four genera have to date not been associated with human disease [2]. Together with the recently identified Bombali ebolavirus (Bombali virus, BOMV) [3], the *Ebolavirus* genus is comprised of six member species: *Zaire ebolavirus* (Ebola virus, EBOV), *Sudan ebolavirus* (Sudan virus (SUDV), *Tai Forest ebolavirus* (Tai Forest virus, TAFV), *Bundibugyo ebolavirus* (Bundibugyo virus, BDBV), and *Reston ebolavirus* (Reston virus, RESTV) [2]. All species except BOBV and RESTV have been associated with ebolavirus-like disease in humans, with EBOV being the major species involved with human disease since the first of two non-related ebolavirus outbreaks (involving EBOV and SUDV) [4] in 1976 (based on current nomenclature, 'ebolavirus' relates to the genus comprised six species: EBOV SUDV, TAFV, BDBV, BOBV, and RESTV). Since this time, human ebolavirus outbreaks have become an ever more frequent occurrence, which is thought to be driven by human activity including deforestation, bushmeat hunting and climate change [4–6]. The world's second largest EBOV outbreak was recently declared over in the Democratic Republic of Congo (DRC) on the 25th of June, 2020 after nearly 2 years and 2,299 deaths [7]. A smaller unrelated outbreak in western DRC also recently ended on the 18th of November, 2020 [8].

Small geographically isolated outbreaks have historically been controlled by implementation of public health measures and alterations in societal behavior. However, the 2013–2015 epidemic in West Africa, which resulted in over 11,000 deaths, showed the substantial impact of EBOV emergence into a heavily populated region with a mobile population and low healthcare infrastructure [9]. It has been suggested that the healthcare infrastructure in these west African countries involved in the epidemic may have been particularly weak even compared to other African countries due to a lower incidence of HIV/AIDS having resulted in a relatively low level of investment in health care [9,10]. The recent 2018–2020 DRC epidemic showed the additional problems associated with civil unrest on EBOV control, where the frequency of conflict events such as attacks on Ebola Treatment Centers affected EBOV control measures such as contract tracing and vaccination. In a recent study, Wells et al (2019) showed the impact of such violence, with increases in the effective reproductive number (R_E) of

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Article highlights

- Lack of preparedness with Ebola virus (EBOV) parallels experience with emergence of other highly pathogenic viruses in terms of development of effective prophylactic and therapeutic, low cost pharmaceutical intervention.
- Investment to preemptively develop such effective and low-cost prophylactic and therapeutic interventions against families of viruses with high potential for emergence and societal impact following emergence such as ebolaviruses and coronaviruses should be made a high priority.
- Inhibitory activity of host-directed drugs observed *in vitro* during repurposing have *not*, to date, translated into *in vivo* efficacy against EBOV in more complex preclinical animal models such as non-human primates (NHPs), and clinical trials in humans.
- Direct acting, and especially monoclonal antibody-based, EBOV drugs show the greatest translational potential for efficacy in NHPs and humans.
- Although approved, the monoclonal-based treatment Inmazeb™ (Regeneron) shows room for improvement, in terms of impact on EBOV-associated disease and mortality and cost. Combination with other direct-acting drugs may be one way to increase effectiveness.

This box summarizes key points contained in the article.

EBOV (observed by number of secondary cases) correlating closely in time with conflict events [11].

In addition to humans, ebolaviruses cause severe disease and high mortality in other great ape species, and EBOV has been associated with significant die-offs of gorillas and chimpanzees in the wild [12,13]. Bats are regarded as the most probable reservoir species for ebolaviruses [14], with spillover believed to occur either directly from bats into humans, or through handling of infected gorilla and chimpanzee carcasses [4]. Different species of fruit bats have long been regarded as the most probable reservoir species involved in ebolavirus zoonotic transmission [15–17]. However, paralleling the zoonotic source of the present SARS-CoV-2 pandemic [18], most evidence supports the role of a small bat in zoonotic emergence during the 2012-2015 EBOV epidemic [19]. The recent identification of BOBV in a similar species of insectivorous bat in Sierra Leone [3], further implicates diverse bat species in precipitating human ebolavirus outbreaks.

1.1. Ebola virus disease (EVD)

Due to a lack of consistent association of hemorrhagic symptomology with infection, Ebola hemorrhagic fever (EHF) was renamed EVD during the 2013–2015 West Africa epidemic. Due to its relatively higher association with human outbreaks (as well as wild ape disease), most is known about EBOV compared to other ebolaviruses, and therefore EBOV will serve as the primary focus of this review. Characteristics of disease associated with EBOV infection result both from direct as well as indirect mechanisms. EVD is characterized by an initial viral prodrome and febrile illness (headache, myalgia, nausea and vomiting), followed by capillary leakage and hemorrhage, which progresses in severe cases to a septic-shock like syndrome with disseminated intravascular coagulation (DIC) and multiorgan failure [20]. EBOV replicates in a wide variety of different cell types (macrophages, dendritic cells (DCs), endothelial cells, hepatocytes and fibroblasts) during infection [21], and the biology of EBOV within these different cells accounts, in large part, for characteristics of EVD.

Following initial infection, typically through a breach in the skin, mucosal exposure, or through direct inoculation into the blood system (i.e. through a needlestick injury or use of a contaminated needle), professional antigen-presenting cells, primarily DCs, and macrophages, serve as the initial site of EBOV replication [22]. Both cell types are highly permissive to EBOV, but the virus affects these two cell types in very different ways. In macrophages, induction of high levels of inflammatory cytokine expression results in the characteristic 'cytokine storm' associated with EVD [23,24]. The effect of this systemic deluge of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 1 (IL-1), and IL-6 is believed to account for much of the indirect effects of EBOV infection such as vascular leakage, hypovolemic shock and, at least in part, lymphocyte apoptosis [21]. Macrophages are also thought to play a key role in promoting virus dissemination to other tissues. In contrast to the effect on macrophages, DC infection is associated with the marked suppression of cytokine expression and induction of a state

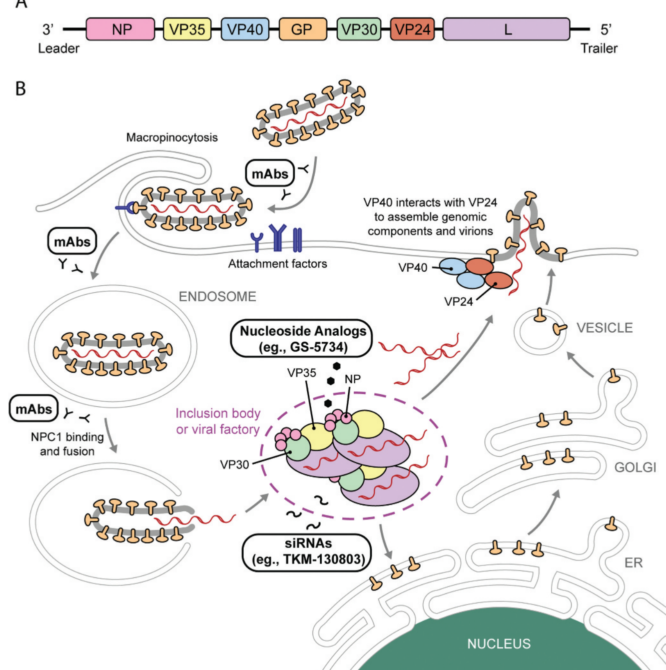

Figure 1. (A) EBOV genome structure. EBOV has a non-segmented, linear, single-stranded negative-sense RNA genome starting with a 3' leader followed by seven genes in the order nucleoprotein (NP), virion protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24 and RNA-dependent RNA polymerase (L) and ending with a 5' trailer. (B) EBOV replication cycle. EBOV mainly enters the cell through macropinocytosis following binding to attachment factors. In the endosome, the EBOV GP binds to its receptor Niemann-Pick C1 (NPC1) and initiates fusion with the endosome membrane to release the genome into the cytoplasm. Transcription and replication occurs in inclusion bodies (viral factories) involving the ribonucleoprotein complex consisting of the genomic RNA, NP, VP35, VP30 and L. Encapsidated genomes move to the plasma membrane and interact with VP24 and VP40. GP traffics through the endoplasmic reticulum (ER) via the Golgi complex to the cell plasma membrane where particle maturation and release occurs. The target sites of promising drugs such as monoclonal antibodies (mAbs), nucleoside analogs and siRNA are indicated.

of functional impairment [23,25]. Given the critical role of DCs in activation of the adaptive immune system, this inhibitory effect on DC function is thought to play a major role in the delay in EBOV-specific adaptive immune response seen during infection, especially in severe disease [21].

Later stages of EVD are associated with infection of additional cell types. Infection of endothelial cells further contributes to the cytokine storm by inducing cytokine expression from these cells [26]. Although EBOV does not infect lymphocytes, the virus induces large-scale bystander apoptotic death of these cells – via intrinsic and extrinsic pathways involving proinflammatory cytokines – which results in further suppression of the adaptive immune response toward the virus [21,27–29]. In patients that survive this viral onslaught and recover, 'immune privileged' cells of the central nervous system (including the eye) and testes may serve as sites of virus persistence providing a continual site for virus reactivation and potential spread [30].

1.2. EBOV Replication

Figure 1 depicts the EBOV replication cycle. EBOV entry is a complex multistep process culminating in cysteine protease–mediated removal of two major extracellulary exposed regions of the viral surface glycoprotein complex [31,32], which results in exposure of the EBOV glycoprotein (GP) receptor-binding domain (RBD) in the late endosomal/lysosomal compartment [33]. This exposure of the RBD enables binding of EBOV GP to Niemann-Pick C1 (NPC1), a multi-pass transmembrane endosomal cholesterol transporter, which is followed by fusion of virion and endosomal membranes and release of the viral genome into the cytoplasm. Although binding to its internal NPC1 receptor appears to be a common feature of EBOV infection in *all* cell types – to a level that NPC1 knock-out mice are completely resistant to EBOV replication [34] – substantial cell-type differences exist at earlier stages of the entry process, notably at the level of initial attachment and uptake of the virus into endosomes.

Initial attachment of EBOV to the cell surface occurs through interactions of N- and O-linked glycans on the heavily glycosylated EBOV GP molecule with multiple C-type lectins (CLECs) that are differentially expressed across a variety of cell types. EBOV attachment also occurs through an interaction of phosphatidylserine (PtdSer – a universal marker of apoptotic bodies) within the virus membrane with PtdSer receptors on the cell surface. Similar to CLEC-based attachment, the specific PtdSer receptors involved in EBOV infection as well as the downstream signaling pathways and mechanism of virus uptake into the endosomal compartment (primarily macropinocytosis, but also caveolin- or clathrin-dependent endocytosis, phagocytosis) have been shown to differ considerably between different cell types [33,35–37]. How EBOV entry compares across key cell types during infection *in vivo* is unclear.

Replication is initiated upon release of the viral ribonucleocapsid (RNP) into the cytoplasm. Primary transcription results in production of viral mRNAs initiated through incoming RNP components from the viral infection, thereby supporting secondary transcription of viral mRNAs and genome replication via a full-length positive-sense antigenome [38]. At approximately 19 kilobases in length, filovirus genomes contain seven linearly arranged genes encoding the nucleoprotein (NP), viral protein 35 (VP35), VP40, GP, VP30, VP24, and the RNA-dependent RNA polymerase (L; RdRp) [39]. EBOV genome transcription and replication is facilitated by the RNP proteins NP, VP35, VP30, and L in intracellular inclusion bodies [40,41]. In the case of ebolaviruses and most likely cuevaviruses, transcriptional editing by the L protein produces GP gene-specific mRNA-species encoding different forms of the glycoprotein: virion-associated $GP_{1,2}$ and two soluble glycoproteins, sGP and ssGP [42]. Over the years, several host factors have been identified to play a role in EBOV replication and transcription such as DNA topoisomerase I (TOP1) [43], the RNA-binding protein Staufen [44] and the RNA splicing and export factors NXF1 and DDX39B [45]. Host factors are also involved in the regulation and balance of replication and transcription via the phosphorylation status of VP30 through the host cell phosphatase PP2A, which is recruited to RNPs by interaction with EBOV NP and the host phosphatase PP1 [46–48]. During the late stages of RNA synthesis VP24, through interaction with the RNP complex, likely mediates condensation of the RNP complexes into replication- and transcription-inactive but packaging-competent RNPs [49,50].

RNPs are subsequently transported to the cell surface in an actin-dependent manner [51,52]. In parallel, the matrix protein VP40 is also transported to the cell surface, where it interacts with cellular trafficking system components such as actin and microtubules [53–56]. The filovirus GP moves to the cell surface through the secretory pathway, where it is post-translationally modified by O- and N-linked glycosylation [57] and furin cleavage into the mature GP_1 and GP_2 subunits [58]. Finally, VP40 coordinates virion assembly and budding at the plasma membrane supported by host factors such as those of the endosomal complex required for transport (ESCRT) and ubiquitin ligases, which interact with VP40 through its late-domain motifs [59–62]. $GP_{1,2}$ was shown to facilitate the trafficking of host scramblases to sites of virion budding, thereby enhancing exposure of PtdSer on the outer envelope of virions for binding to PtdSer receptors such as TIM-1 during entry [63].

1.3. Animal models

The U.S. Food and Drug Administration (FDA) has implemented the animal rule allowing licensing of countermeasures based on efficacy data in animal models in combination with animal and human safety trials [64]. Multiple common laboratory animal species serve as animal disease models for EBOV. Traditionally, drug candidates are initially screened in rodent models (mouse, hamster and guinea pig [65]). Unfortunately, clinical EBOV isolates typically need serial adaptation to a rodent species to produce disease with uniform lethality. Mouse models mainly utilize common laboratory strains, which do not always closely mimic manifestations and progression of human EVD. A proportion of promising *in vitro* drug compounds show efficacy in mouse models, but the predictive value for efficacy in NHPs and humans is relatively low (Tables 1 and Table 1 Supplemental) [66,67]. Hamster and guinea pig models are less often utilized for countermeasure

efficacy testing mainly due to more difficult handling in biocontainment and lack of immunological tools. However, their predictive value for countermeasure efficacy in NHPs and humans is higher. NHPs, especially cynomolgus and rhesus macaques, are considered the gold standard animal models for EBOV due to similar disease presentation as EVD in humans [66]. Compared to cynomolgus macaques, rhesus macaques display a slightly prolonged time to death upon EBOV infection allowing for an extended window of intervention making them the preferred NHP model for drug efficacy studies. The ferret is a relatively new animal disease model for EBOV with even more restrictive limitations than for hamsters and guinea pigs, and an unknown predictive value for efficacy in NHPs and humans. Today, most drug compounds developed for EBOV have gone through the rodent screening and NHP confirmatory preclinical pathway prior to use in clinical trials (Supplemental Tables 1 and 2) [68].

2. EBOV therapeutics

It has been estimated that the average drug takes 10 years to bring to market and costs more than 2 USD billion, with much of the cost attributed to the high drug failure rate relating to efficacy and safety [87,88]. Repurposing of drugs that are either already commercially approved, or are investigational but have substantial preclinical pharmacokinetic and toxicological data supporting movement toward licensure for other clinical diseases, has been proposed as a means to quickly identify interventional drugs for the control of emerging infectious diseases. Repurposing is certainly appealing as it bypasses the time-consuming and expensive toxicological testing with its high risk of failure, and optimization of drug formulation to ensure bioavailability. Repurposing is also particularly appealing for therapeutics against pathogens such as EBOV that have a limited commercial market. However, the extremely small number of drugs that have been successfully repurposed beyond their specific drug class is an indicator of the hidden complexities of this approach [89]. To our knowledge, no drug originally developed for non-viral diseases has been effectively repurposed as an antiviral [90]. In contrast, a number of direct-acting antivirals have been effectively repurposed within their class, but beyond their initially intended virus target.

To establish a comprehensive history of pharmaceutical interventions targeting ebolaviruses, searches were conducted in the PubMed and Web of Science databases. Keywords including 'Ebolaviruses', 'Ebolavirus disease', 'Ebola', 'Marburg', 'Bombali', 'Zaire', 'Sudan', 'Tai Forest', 'Bundibugyo', 'Reston', in combination with 'drug', 'antibody', 'therapeutic', 'treatment', 'pharmaceutical', and 'intervention' were used to identify studies of interest. Results were filtered to include only articles published between 1976 and the present with the latest date of search being conducted on the 24th of October, 2020.

Multiple studies investigating the repurposing of commercially approved drugs have been performed for EBOV. Table 1 and Supplemental Table 1 detail therapeutics that have been repurposed for the treatment of EBOV to the level of testing in a preclinical animal model. Notably, similar to the situation with the present SARS-CoV-2 pandemic, many of these studies were initiated only after a major disease outbreak – in this case, the 2013–2015 West Africa EBOV epidemic. To remove the need for such reactionary responses, it has recently been suggested that investment should be made into development of antiviral agents to provide preemptive tools to control emerging viruses with high potential for societal impact, such as filoviruses and coronaviruses [91]. Such a preemptive strategy that develops through to licensure drugs effective against known individual pathogens, like EBOV or groups of pathogens such as filoviruses or coronaviruses with a history of emergence, has considerable attraction from the present COVID-19 viewpoint.

Prior to preclinical testing, drugs identified through repurposing in *in vitro* screens need also to be assessed based on known clinical *in vivo* pharmacokinetic parameters to ensure therapeutic levels are achievable through normal established dosing regimens. It is also important to critically evaluate drugs for their potential for adverse reactions and difficulties in establishing dosing schedules within the anticipated infected patient setting. A tier-based system for ranking of licensed drugs with inhibitory activity identified against emerging pathogens was recently established, in this case to identify drugs with potential for repurposing against SARS-CoV-2 [92]. Using this system, drug candidates showing *in vitro* inhibitory activity against the virus being targeted can be rapidly triaged based on pharmacokinetic parameters (e.g. whether *in vitro* inhibitory and *in vivo* therapeutic levels are comparable), as well as based on the presence of adverse events not consistent with prophylactic/therapeutic use. The remaining drugs can then be prioritized for testing in preclinical animal models. Remarkably, using this system, only 5 of 56 drugs with *in vitro* inhibitory activity against coronaviruses (representing 3 distinct drug classes) were suitable for onward movement into preclinical trials [92]. As will be detailed below, this experience is consistent with the poor outcome of repurposing drugs for EBOV to date.

Translation from *in vitro* studies to test for potential efficacy *in vivo* must also be cautioned with a number of additional caveats. Even with full commercial licensure, many drugs have a defined and limited life of production for the market, being superseded by newer drugs in class with better safety, efficacy or ease of use profiles. This is particularly common for hepatitis C virus and HIV antivirals. For repurposing, the lack of available drug formulated for high bioavailability that results from such cessation of commercial production can severely impair movement of drugs showing *in vitro* efficacy into preclinical animal models. This caveat clearly removes the 'off the shelf' availability attraction of repurposed drugs.

Aside from the inherent variability of inhibitory activity observed *in vitro* due to differences in assay conditions [90,93], the possibility for cell type-specific differences in characteristics of virus replication also needs to be considered. African green monkey-derived Vero E6 are a common cell type used to screen for drug inhibitors of many viruses, including EBOV. However, infection in these cells will not reflect the behavior of the EBOV within all cell types important for disease *in vivo*. In addition to differences in the entry process, Vero E6 cells are also known to be deficient in multiple aspects of their innate immune response

Table 1. Drug-based therapeutics (macaque challenge model).

Drug	Drug Class	Dose	Species	Challenge	Efficacy (Survival)	In Vitro	Pharmacokinetics and additional Details
GS-5734 (Remdesivir) [188]	Antiviral nucleoside analog	10 mg/kg q24h IV (starting +D3)	Rhesus macaque (high esterase activity in rodents precludes testing)	1000 pfu EBOV Kikwit	Complete (100%, +D3)	EC_{50} 0.021–0.066 µM. Reporter virus assay using EBOV labeled with GFP or GLuc in Huh 7 cells [69,193]	Showed to be inferior with respect to mortality at interim analysis as 1 of 4 treatments in 2018/2019 clinical efficacy trial (NCT03719586), and discontinued[176] Over 144 h post IV infusion, with 225 mg of remdesivir. Remdesivir: C_{max} of 4420 ± 30.1 ng/mL (7.3 ± 0.05 µM) with $t_{1/2}$ of 1.05 (0.96–1.21) h. GS-441,524: C_{max} of 257 ± 30.2 ng/mL (0.88 ± 0.10 µM) with $t_{1/2}$ of 30.6 (29.5–31.1) h. GS-704,277: C_{max} of 315.1 ± 19.5 ng/mL (0.71 ± 0.04 µM) with $t_{1/2}$ of 1.77 (1.39–1.90) h s[70]
BCX4430 (Galidesivir)[184]	Antiviral nucleoside analog	25 mg/kg q12h IM (starting D0); and 100 mg/kg q12h IM (2 loading doses starting either +D2 or +D3) followed by 25 mg/kg q12h	Rhesus macaque	Ebolavirus (dose and species not indicated)	Complete (100%, D0) Complete (100%, +D2) Moderate (67%, +D3)	EC_{50} 11.8 µM. High content image analysis using EBOV Kikiwit in HeLa cells [69,181]	Single-dose of IV delivered 20 mg/kg galidesivir resulted in C_{max} of 20.5 mg/L (77.3µM). $t_{1/2}$ not reported [71]
(1) T-705 (Favipiravir)[200]	Antiviral nucleoside analog	400 mg/kg PO loading (-D3) followed by 200 mg/kg daily (-D2 to +D10; and 250 mg/kg q12h PO loading (D0) followed by 125 mg/kg q12h (starting +D1); and 125 mg/kg q12h PO loading (D0) followed by 75 mg/kg q12h (starting +D1)	Cynomolgus macaque	1000 pfu EBOV Kikwit	Low (17%; 400 mg/kg PO, – D3) None (0%; 250 mg/kg and 125 mg/kg PO, D0)	EC_{50} 281.6 µM. High content image-based cell assay using EBOV Kikwit in HeLa cells [72]	Steady state from twice daily oral dose of 400 mg in humans results in T-705 C_{max} of 30.56 mg/L (194.5 µM) with a $t_{1/2}$ of 4.5 hours. Twice Daily 600 mg oral dose resulted in a steady state C_{max} of 61.50 mg/L (391.4 µM) and a $t_{1/2}$ of 4.5 h [182]
(2) T-705[184],[202]	Antiviral nucleoside analog	250 mg/kg q12h IV loading (– D2) followed by 180 mg/kg or 150 mg/kg q12h (starting – D1); and 200 mg/kg q12h IV loading (–D2) followed by 100 mg/kg q12h (starting – D1)	Cynomolgus macaque	1000 pfu EBOV Gabon 2001	Moderate (60%; 180 mg/kg, – D2) Moderate (40%; 150 mg/kg, – D2) None (0%; 100 mg/kg, – D2)	As above	As above
Sertraline[141]	Antidepressant (CAD)	200 mg q24h (starting – D6)	Rhesus macaque	1090 pfu EBOV/Mak-C05	None (0%, – D6)	IC_{50} 3.13 ± 0.24 µM Luminescence cell viability assay using eGFP-EBOV in Vero E6 cells [69,119]	Giving a once daily 50 mg/day dose of oral sertraline over 30 days resulted in sertraline C_{max} of 142 ± 53 µg/L (0.46 ± 0.17 µM) with a terminal $t_{1/2}$ of 22.4–32.1 h. The metabolite *N*-demethylsertraline achieved a C_{max} of 200 ± 75 µg/L (0.68 ± 0.26 µM) with no reported $t_{1/2}$[73]

Efficacy: Green = high, Yellow = moderate, Orange = low.Drug dosing and challenge: Q = every, IV = intravenous, IP = intraperitoneal, IN = intranasal, IM = intramuscular, PO = oral.Pharmacokinetics: C_{max} = maximum plasma concentration achieved, $t_{1/2}$ = time to half-life.In vitro: IC_{50} = half-maximal inhibitory concentration, EC_{50} = half-maximal effective concentration, ADCC = antibody-dependent cell-mediated cytotoxicity.

pathway [94,95]. Cell type-specific differences may be particularly important for viruses like EBOV that infect a wide diversity of cell types at different stages of disease, and that are highly sensitive to modulation by innate immune response pathways [96–98].

2.1. Conventional drug screening

EBOV inhibitory drugs can be broadly divided into two categories: (i) indirect acting, host-directed and (ii) direct acting, virus-directed. Over the past two decades, myriad drugs have been tested for their inhibitory capacity against EBOV [99–105]. Multiple studies have used low throughput, information-guided approaches to identify drugs for screening of EBOV inhibitory activity using critical evaluation of potential drug candidates based among other criteria on understanding of mechanism, prior demonstration of antiviral activity, and drug availability [106–117]. For example, in response to the West Africa 2013–2015 EBOV epidemic, Dowall et al. (2016) [118] selected from a panel of antimicrobial agents, 18 drugs for repurposing against EBOV. The drugs were selected based either on earlier published evidence supporting an ability to inhibit EBOV replication *in vitro*, or on an anticipated high potential for inhibitory activity against the virus. Half of these compounds were removed from further study due to an inability to produce an *in vitro* inhibitory effect on EBOV replication. Of the remaining compounds, three were selected for further characterization in the guinea pig EBOV challenge model. However, one showed high-level *in vivo* toxicity preventing further analysis, and neither of the two remaining agents resulted in a statistically significant impact on guinea pig survival or EBOV disease (see Tables 1 and Table 1 Supplemental) [118]. These studies highlight the frequent lack of efficacy when translating from *in vitro* inhibition studies into *in vivo* preclinical models, especially for indirect acting host-directed therapeutics that can also be associated with significant toxicity.

2.2. High-throughput screening (HTS)

Conventional drug screening approaches have been augmented by development of high-throughput screening (HTS) assays [119–124], which has increased the rate at which drugs can be screened for *in vitro* inhibitory activity against EBOV. To enable higher throughput and more rapid screening than is possible when using more conventional methods of EBOV quantitation, a number of laboratories have designed recombinant systems to either (i) more easily enable quantitation under BSL-4 high containment conditions, or (ii) enable analysis under lower levels of containment. For example, Towner et al. developed a recombinant EBOV containing green fluorescent protein (GFP) inserted within the viral genome [125]. The utility of the virus for screening of drugs *in vitro* under BSL-4 containment was initially demonstrated using interferon-alpha in proof-of-concept studies. Using GFP EBOV-based assays (as well as lower containment pseudotype-based entry screens, see below), a series of HTS studies have been used to screen libraries of molecular probes and commercially approved drugs [117,119,120,126].

Additional strategies have been developed to enable HTS-based screening, at least initially, under lower or no containment level. These HTS analyses are comprised of both bioinformatic-, and biologically based assays or combinations of the two approaches [88,127–130]. As many of these screens rely on computer modeling of small-molecule interactions with EBOV proteins or essential cellular proteins, they are necessarily focused on well-characterized interactions and resolved structures. Machine learning-based training using results from earlier *in vitro* screens [120,126] has been used as an alternative bioinformatics-based approach for identification of EBOV inhibitors. One such large virtual screen identified three potentially active compounds, which were then confirmed experimentally *in vitro* using a GFP-labeled EBOV [131]. All three candidates were shown to have high levels of *in vivo* inhibitory activity in the mouse EBOV challenge model (Supplemental Table 1) [132–134].

To increase usability of HTS for biologically based filovirus drug screening, Uebelhoer et al. established an HTS based on a supernatant luciferase concentration readout using a low containment mini-genome EBOV luciferase system in a T7 polymerase baby hamster kidney cell line as an initial screen, followed by recombinant luciferase-expressing EBOV at high containment using a variety of different cell types [135]. The potential utility of this dual system was further optimized by use of a nonfunctional RdRp EBOV control to confirm RdRp-dependent inhibition in the mini-genome system [136]. Screening systems based on virus-like particles (VLPs) and pseudotyped viruses have been developed as an alternative HTS strategy to identify EBOV entry inhibitors without the need for high containment [121,137,138]. These have also been used as components of larger HTS screens, which can also provide further mechanistic insight into the inhibitory activity of the drugs [117,119,120,122,126].

2.3. In vivo studies

Both host-directed and direct-acting agents have been tested in preclinical animal models. However, only direct-acting inhibitors that target either virus replication at the level of the RdRp (nucleoside analogs) or entry by binding to GP (monoclonal antibodies; mAbs) have shown any efficacy in the NHP EBOV model, considered the most stringent preclinical model before translation of drugs and vaccines into humans. Consistent with performance in NHPs, only direct-acting antivirals have shown any efficacy in controlled human clinical trials.

2.3.1. Host-directed antiviral agents

A number of drugs representing a few key classes that target cellular pathways involved in EBOV replication have been identified through *in vitro* analyses to inhibit EBOV replication. These include a number of inhibitory lectins, which are believed to function at early, attachment stages of the infection process. Cationic amphiphilic drugs (CADs) and structurally related amphiphiles represent a second large group of drugs identified with EBOV inhibitory activity, and include antimalarial amino-quinolones, selective estrogen receptor molecules (SERMS), antipsychotics and antidepressants

among others. These drugs are frequently identified as host-directed antivirals having an *in vitro* inhibitory activity against EBOV as well as many other viruses (Table 1 and Supplemental Table 1) [139]. Structurally, CADs are composed of a hydrophobic ring structure linked to a hydrophilic moiety with an ionizable amine group. Mechanistically, they become trapped within late endosomes/lysosomes due to protonation of their amine groups, and are believed to primarily exert their antiviral function through an effect on endosomal/lysosomal pathways. The calcium channel blocker CADs appear to function at late endosomal stages of virus fusion [139].

Translation of host-directed drugs with *in vitro* inhibitory activity against EBOV to efficacy in preclinical animal models has been extremely poor, with an associated increasing frequency of failure in efficacy as one moves through increasingly stringent animal models from mice through guinea pigs to NHPs (Table 1 and Supplemental Table 1). Such failures of translation into preclinical models do not necessarily always equate with a demonstrated inability of the drug to prevent EBOV disease, but rather can also result from unpredicted toxicity of the drug, or an inability to reach expected inhibitory levels in the preclinical model (Supplemental Table 1) [126,140].

The case of the CAD sertraline (Zoloft) provides a good example of a host-directed antiviral to emphasize the repeated and consistent failure of indirect-acting drugs to translate initial promising results from *in vitro* studies to *in vivo* preclinical models. As the only host directed antiviral agent to have been moved from *in vitro* analyses to be tested in both rodent and NHP models, sertraline also highlights the failure of drugs with high inhibitory activity in mice to translate to efficacy in more stringent guinea pig and NHP models. Sertraline was originally identified through HTS as having low micromolar *in vitro* inhibitory activity against EBOV in a number of different cell types [119,137]. Additional analysis revealed broad *in vitro* activity against multiple filoviruses [119]. Mechanistically, sertraline was shown to function at a late viral entry step after transport to NCP1+ endosomes consistent with its CAD nature [119]. These results led to assessment of efficacy in the mouse model wherein the drug was shown to provide high (70%) protection against high-dose challenge of mouse-adapted EBOV (Supplemental Table 1) [119]. Based on these data, high-dose sertraline was assessed as a prophylactic against EBOV infection and disease in the stringent NHP rhesus macaque EBOV model (Table 1). Although earlier single dosing studies indicated that plasma levels in the NHPs were below *in vitro* inhibitory levels, the authors indicated that the multiple dosing regimen used would have brought plasma levels to within range. The results of this study are notable in terms of the lack of effect of sertraline on *any* parameter of EBOV infection, including clinical disease and EBOV viremia in the NHP model compared to controls, even given the drug's demonstrated high potency in multiple cell types *in vitro* and high efficacy in the mouse EBOV challenge model [141]. This study also emphasizes the importance of publication of results from well-controlled studies even when negative.

Although sertraline was not assessed in any subsequent clinical trial, a phase I/II clinical trial (EMERGENCY Amiodarone Study Against Ebola; EASE) (NCT02307591) using another host acting CAD, amiodarone, was moved to the recruitment phase during the West Africa EBOV epidemic [142]. Similar to sertraline, amiodarone had shown potent *in vitro* inhibitory activity [126]. Although this CAD had not been tested in NHPs, later published studies showed it to have low efficacy in the mouse model (Supplemental Table 1) [126]. The clinical trial, led by an Italian non-governmental organization [143], raised concerns at the time regarding the drug's associated toxicity [144]. The trial was never started, citing insufficient number of new EVD cases in the waning epidemic [145]. The poor translation of host-directed antivirals with potent *in vitro* activity to *in vivo* efficacy is not completely understood, but is presumably due to multiple factors including *in vitro* assays being unable to completely model replication that is relevant to cell types involved in EBOV disease *in vivo*, the presence of redundant pathways of virus biogenesis, and possible mismatches between *in vitro* inhibitory doses and *in vivo* therapeutic drug levels.

2.3.2. Direct-acting antiviral agents

In contrast to host-directed drugs, a number of direct-acting antivirals have proved more successful against EBOV, as well as other filoviruses. These consist of either mAbs against EBOV GP, or drugs that target the EBOV RdRp (nucleoside analogs). Due to their nature, mAbs have necessarily been developed as an intervention specific to EBOV. In contrast, all currently identified RdRp-targeting drugs have been developed against one particular virus or group of viruses, and then repurposed against EBOV. In general, presumably due to superior potency, higher achievable plasma levels without associated toxicity, and longer half-lives, mAbs have shown themselves as superior in efficacy to nucleoside-based inhibitory drugs against EBOV in all preclinical models and humans. Nucleoside analogs, although less efficacious against EBOV, have a greater capacity for broader application to the treatment of other filoviruses, and even beyond to other families of viruses commonly associated with emergence. Depending on the drug, they also have potential for oral administration outside the hospital setting, for example, following high-risk exposure.

2.3.2.1. Antibodies. The development of treatment options for filovirus infections started shortly after initial emergence of ebolaviruses (in this case, EBOV and SUDV) in 1976. However, before the West African EBOV outbreak efficacy had only been shown in preclinical animal studies. Clinical trials of mAb treatment options first began during the 2013–2015 West African EBOV outbreak. Prior to this time, the use of antibody-based approaches to treat EBOV infection had been controversial. The first documented application of passive antibody therapy to treat an ebolavirus infection occurred in 1976. A UK laboratory worker, who had a needle stick exposure with a yet unidentified ebolavirus specimen, was initially treated with anti-EBOV convalescent serum followed by a second dose of anti-SUDV convalescent serum [146]. The patient survived; however, the contribution of antibodies to survival remains unknown as the patient had also received interferon aside of advanced intensive care.

Table 2. Antibody-based therapeutics (macaque challenge model).

Drug	Drug class	Dose	Species	Challenge	Efficacy (Survival)	In Vitro	Pharmacokinetics & Additional Details
REGN-EB3 [74, 75]	Cocktail of fully humanized mAbs produced in VelocImmune mice. 1:1:1 mix of REGN3470, REGN3471 and REGN3479 (see below). Produced in low fucose CHO cells	50 mg/kg (total mAb) IV administered at (i) +D5, +D8, and +D11; and (ii) at +D5 and +D8, or at 150 mg/kg as a single dose at +D5; and (iii) 150, 100, 50, or 10 mg/kg as single dose at +D5	Rhesus macaque	1000 pfu EBOV Kikwit	(i) High (92%; 50 mg/kg, +D5, +D8 and +D11) (ii) Complete (100%; 50 mg/kg, +D5 and +D8) High (80%; 150 mg/kg, +D5) (iii) High (89%; 150 mg/kg or 100 mg/kg, +D5) High (77%; 50 mg/kg, +D5) Moderate (44%; 10 mg/kg, +D5)	IC_{50} 0.39 nM (pseudotype neutralization); EBOV infectivity IC_{50} not tested; FcγRIIIa signaling EC_{50} 1.7 nM [75]	Human PK data: Linear with mean $t_{1/2}$ ranging from 21.7 to 27.3 days. C_{max} not indicated. Showed superiority with respect to mortality at interim analysis as 1 of 4 treatments in 2018/19 clinical efficacy trial (NCT03719586)[176]
mAb 114 [74,76,170]	Single human mAb produced in CHO cell line	50 mg/kg IV administered at +D1, +D2, and +D3; and 50 mg/kg IV administered at +D5, +D6, and +D7	Rhesus macaque	1000 pfu EBOV Kikwit	Complete (100%; +D1 or +D5 start)	IC_{50} 0.6 nM (pseudotype neutralization)[170]; EBOV infectivity IC_{50} < 1.33 nM; ADCC EC_{50} 0.2nM	Human PK data: Linear with mean $t_{1/2}$ 24.2 (±1.8) days [176] Mean C_{max} for 50 mg/kg dose 1961 μg/ml (13 μM) Macaque PK data: Linear kinetics with 7 to 15 day $t_{1/2}$ 205 Showed superiority with respect to mortality as 1 of 4 treatments in 2018/19 clinical efficacy trial (NCT03719586)[176]
mAb 100 + mAb 114 [170]	Human mAb cocktail	50 mg/kg (total mAb) IV administered at +D1, +D2, and +D3	Rhesus macaque	1000 pfu EBOV Kikwit	Complete (100%)	Not determined for cocktail. For mAb100: IC_{50} 0.4 nM (pseudotype neutralization); EBOV infectivity IC_{50} <1.33 nM; ADCC EC_{50} 0.2 nM. See above for mAb 114	As above (mAb 114). Not further developed as a mAb cocktail
(1) ZMapp1 (ZMapp™) [167]	Human chimeric mAb cocktail of humanized mAb c2G4, c4G7 and c13C6 produced in *N. benthamiana* (tobacco) plants	50 mg/kg IV administered at +D3, +D6, and +D9	Rhesus macaque	2512 pfu EBOV Kikwit	Complete (100%)	Not determined for cocktail. Individual EBOV infectivity IC_{50}: c2G4 1.25 nM (0.187 μg/ml), c4G7 0.56 nM (0.084 μg/ml), and c13C6 0.2 nM (0.033 μg/ml) [169]	Human PK data not yet available (NCT02389192) [145,182] Showed to be inferior with respect to mortality at interim analysis as 1 of 4 treatments in 2018/19 clinical efficacy trial (NCT03719586), and discontinued [176]
(2) ZMapp1 (ZMapp™) [167]	As above	50 mg/kg IV administered starting at either +D3, +D4, or +D5 followed by 2 more doses q72h	Rhesus macaque	628 pfu EBOV Kikwit	Complete (100%; starting +D3, +D4 and +D5)	As above	As above

Table 2. (Continued).

Drug	Drug class	Dose	Species	Challenge	Efficacy (Survival)	In Vitro	Pharmacokinetics & Additional Details
ZMapp2 [167]	Human chimeric mAb cocktail of c2G4, c1H3, and c13C6	50 mg/kg IV administered at +D3, +D6, and +D9	Rhesus macaque	2512 pfu EBOV Kikwit	High (83%)	Not determined for cocktail. Individual EBOV infectivity IC_{50}: c2G4 1.25 nM (0.187 µg/ml), c13C6 0.2 nM (0.033 µg/ml,[169], and not quantified for c1H3. [77] Mouse mAb parent of c1H3 EBOV infectivity IC_{50} 180 nM (27.6 µg/ml) compared to 0.93 nM (0.139 µg/ml) for c2G4 parent mouse mAb [78]	Not further developed *in lieu* of ZMapp1 (ZMapp™)
MIL77 [79,182]	Human chimeric mAb cocktail comparable to ZMapp™ containing variable regions of mAb c2G4 (MIL77–1), c4G7 (MIL77–2) and c13C6 (MIL77–3) reengineered for enhanced production in CHO, and with low fucosylation	20 mg/kg IV administered on +D3, +D6, and +D9	Rhesus macaque	1000 pfu EBOV Kikwit (isolate 199510621)	High (80%)	Not determined for cocktail. Individual EBOV infectivity IC_{50}: MIL77–1 1.23 nM (0.185 µg/ml), MIL77–2 0.99 nM (0.149 µg/ml), and MIL77–3 0.25 nM (0.037 µg/ml) [169]	WHO recommend completion of ZMapp™ clinical analysis prior to enrollment into clinical studies (now completed, see above) [80,176] PK parameters assessed in cynomolgus macaques: Mean $t_{1/2}$ 6.7 (±1.6) days. Mean C_{max} for 150 mg/kg dose 3203.8 (±323.4) µg/ml (21.4 µM) [169]
MIL77E [169]	Human chimeric mAb cocktail containing MIL77–1 and MIL77–3 (2:1 ratio)	50 mg/kg of cocktail IV administered on +D3, +D6, and +D9	Rhesus macaque	1000 $TCID_{50}$ EBOV Makona (Mak-C05)	Complete (100%)	Not determined for cocktail. See above for individual mAbs	As above
MBP134[AF][177]	mAb cocktail (1:1) of afucosylated mAbADI-15,878 and mAbADI-23,774 produced in in *N. benthamiana*	Administered as a single IV dose of 25 mg/kg at +D4, or a dose of 50 mg/kg and 25 mg/kg at +D4 and +D7, respectively	Rhesus macaque	988 pfu EBOV Kikwit-199510621	Complete (100% single and double)	IC_{50} < 1 nM (pseudotype neutralization) against all 5 EBOV species and BOMV [81]	Human PK data not available
MBP134[AF][177]	As above, but produced in CHOK1-AF	Administered as a single IV dose of either 25 mg/kg or 7.5 mg/kg on +D5	Rhesus macaque	1750 pfu SUDV Boniface	Complete (100% 25 mg/kg and 7.5 mg/kg)		As above
MBP134[AF][177]	As above	Administered as a single IV dose of 25 mg/kg on +D7	Cynomolgus macaque	938 pfu BDBV	High (83%)		As above

Table 2. (Continued).

Drug	Drug class	Dose	Species	Challenge	Efficacy (Survival)	In Vitro	Pharmacokinetics & Additional Details
mAb EBOV-520 + EBOV-548 [82]	Human mAb cocktail (1:1) of mAb EBOV-520 engineered for low Fc binding by fusion to IgG1-LALA Fc and EBOV-548 containing fully functional Fc. Both mAbs produced in CHO cells.	30 mg/kg IV administered at +D3 and +D6	Rhesus macaque	1000 pfu EBOV Kikwit (variant 199510621)	Complete (100%)	Not determined for cocktail. Individual mAb virus infectivity $IC_{50:}$ EBOV-520 for EBOV 12.0–38.2 nM (1.801– 5.738 µg/ml); BDBV: 1.33 nM (0.2 µg/ml); SUDV: 11.2 nM (1.672 µg/ml)[82,83] EBOV-548 for EBOV 10.6 nM (1.6 µg/ml); BDBV: 15.0 nM (2.26 µg/ml); SUDV: Not detected [82]	Human PK data not available. In rhesus macaques, 30 mg/kg IV administered at +D3 and +D6 results in C_{max} range of 0.83 –3.1 µM (125– 460 µg/ml)
ZMAb [164]	Mouse mAb cocktail of mAb2G4, mAb4G7, and mAb1H3	25 mg/kg IV administered at either +D1, +D4, and +D7, or +D2, +D5, and +D8	Cynomolgus macaque	1000 pfu EBOV Kikwit	Complete (100%; starting +D1) Moderate (50%; starting +D2)	Not determined for cocktail. For individual mAb EBOV infectivity IC_{50}: 2G4 0.93 nM (0.139 µg/ml), 4G7 0.90 nM (0.135 µg/ml), and 1H3 184 nM (27.6 µg/ml) [78]	Further developed into ZMapp™
(1) MB-003$_{RAMP}$ [165]	Human chimeric mAb cocktail of c6D8, h13F6, and c13C6 produced in *N. benthamiana*	16.7 mg/kg IV administered at +1 h, +D4, and +D8	Rhesus macaque	1000 pfu EBOV Kikwit	Complete (100%)		Further developed into ZMapp™
Protein A-concentrated convalescent polyclonal IgG [163]	Pooled gamma-irradiated protein A concentrated IgG from rhesus macaques vaccinated with viral vectors that survived EBOV challenge	80 mg/kg IV administered on +D2, +D4, and +D8	Rhesus macaque	1000 pfu EBOV Kikwit	Complete (100%)	EBOV infectivity IC_{50} approx. 53 nM (8 µg/ml)[163]	Established capacity for passive antibody post-exposure therapy to provide high level protection in macaque model
MB-003$_{CHO}$[165]	Human chimeric mAb cocktail of c6D8, h13F6 and c13C6, produced in Chinese hamster ovary (CHO) cells	50 mg/kg IV administered at +1 h, +D4, and +D8	Rhesus macaque	1000 pfu EBOV Kikwit	Moderate (50%)		See above
MB-003$_{RAMP}$[165]	Human chimeric mAb cocktail of c6D8, h13F6, and c13C6 produced in *N. benthamiana*	16.7 mg/kg IV administered at +D1, +D5, +D8, and +D10; and +D2, +D6, +D8, and +D10	Rhesus macaque	1000 pfu EBOV Kikwit	Moderate (67%; +D1 start) Moderate (67%; +D2 start)		See Above
mAb c13C6[167]	Single human chimeric mAb	50 mg/kg IV administered at +D1, +D4, and +D7	Rhesus macaque	1000 pfu EBOV Kikwit	Low (33%)	EBOV infectivity IC_{50} 0.2 nM (0.033 µg/ml)[169]	Further developed into component of ZMapp™

Table 2. (Continued).

Drug	Drug class	Dose	Species	Challenge	Efficacy (Survival)	In Vitro	Pharmacokinetics & Additional Details
mAb h13F6[167]	Single human chimeric mAb	50 mg/kg IV administered at +D1, +D4, and +D7	Rhesus macaque	1000 pfu EBOV Kikwit	Low (0%)	Non-neutralizing. Based on original mAb 13F6 [158]	Component of MB-003
mAb c6D8[167]	Single human chimeric mAb	50 mg/kg IV administered at D +D1, +D4, and +D7	Rhesus macaque	1000 pfu EBOV Kikwit	Low (0%)	Neutralizing but requiring complement. EBOV infectivity IC_{80} 42 nM (6.25 µg/ml). Based on original mAb 6D8 [158]	Component of MB-003
MB-003$_{RAMP}$[167]	Human chimeric mAb cocktail of c6D8, h13F6, and c13C6 produced in *N. benthamiana*	50 mg/kg IV administered at +D1, +D4, and +D7	Rhesus macaque	1000 pfu EBOV Kikwit	Low (33%)		Further developed into ZMapp^{TM}
mAb KZ52 [162]	Single human mAb	50 mg/kg IV administered at –D1 and +D4	Rhesus macaque	1000 pfu EBOV Kikwit	Low (25%)	EBOV infectivity IC_{50} 2 nM [84]	Macaque mAb levels at +D4 range 1.3 µM (200 µg/ml) to 2.7 µM (400 µg/ml) [162] Not further developed
mAb ch133 + mAb ch226 [86]	Human chimeric mAb cocktail version of mouse mAb 133 and 226 [85]	50 mg/kg (1:1) IV administered at –D1, +D1, and +D3	Rhesus macaque	1000 pfu EBOV Kikwit	Low (33%)	Not determined for cocktail. For individual mAbs: ch133 EBOV infectivity IC_{50} 10.6 nM (1.6 µg/ml), and ch226 EBOV infectivity IC_{50} 14 nM (2.1 µg/ml) [86]	Not further developed. Half-life in rhesus macaques 3–4 days. >0.5 µM (75 µg/ml) maintained +D1 to +D5. Rapidly decreased in animals that succumbed to infection at +D8. In single survivor >0.33 µM (50 µg/ml) until +D11, after which host IgG increased [86]
Convalescent hyperimmune blood transfusion [148]	Hyperimmune (100,000 ELISA titer) blood transfusion from EBOV-immune rhesus macaques	6 ml/kg IV administered D0, or D0 and +D3	Rhesus macaque	EBOV (strain and dose not indicated)	None (0%)	Not determined	Not further developed

Efficacy: Green = high, Yellow = moderate, Orange = low.Drug dosing and challenge: Q = every, IV = intravenous, IP = intraperitoneal, IN = intranasal, IM = intramuscular, PO = oral.Pharmacokinetics: C_{max} = maximum plasma concentration achieved, $t_{1/2}$ = time to half-life.
In vitro: IC50 = half-maximal inhibitory concentration, EC50 = half-maximal effective concentration, ADCC = antibody-dependent cell-mediated cytotoxicity.

The next use of convalescent serum was documented during the Kikwit EBOV outbreak in 1995 [147]. Eight patients were treated with convalescent whole blood of which seven survived. Despite the treatment success, the role of antibodies was disputed due to multiple other factors that may have influenced the outcome. Given the unsatisfying situation, two NHP studies were performed to address the efficacy of whole blood or serum as a treatment option for EBOV. Interestingly, treatment with homologous convalescent whole blood or pooled homologous convalescent anti-EBOV serum did not protect the animals from lethal EBOV challenge, with not even a delay in disease progression (Table 2) [148,149]. During the 2013–2015 EBOV outbreak, a nonrandomized, comparative study was conducted in West Africa using convalescent plasma. Treatment was not associated with significant improvement in survival [150]. During the same outbreak, convalescent blood products were also used for the treatment of several repatriated EBOV patients. However, the concomitant use of other experimental therapies and advanced intensive care again prevented conclusions on the role of antibodies for patient outcome [151–154].

The first use of passive antibody therapy not derived from convalescent donors was an equine hyperimmune serum studied in the baboon EBOV model [155]. The equine hyperimmune serum completely protected baboons when administered before or up to 1 h after EBOV challenge, but survival rapidly declined when treatment was delayed further. To confirm efficacy of this product, the treatment was evaluated in the EBOV cynomolgus macaque model. Here, only partial success was demonstrated when animals were treated twice beginning shortly after EBOV exposure followed by a second dose 5 days later [156,157]. Despite offering this product to the World Health Organization (WHO), equine hyperimmune serum has never been advanced for human use.

Development of mAbs for ebolavirus began in the early 1990s (Table 2 and Supplemental Table 2) [158]. Clinical failures of some murine mAbs for a variety of different indications [159,160] forested the development of humanized murine mAbs and later fully human mAbs. The first human EBOV mAb was the potent glycoprotein-specific neutralizing KZ52 derived from a survivor of the EBOV-Kikwit outbreak in 1995 [143]. KZ52 demonstrated potent efficacy in the lethal EBOV guinea pig model [161], but failed to protect in the lethal EBOV rhesus macaque model (Table 2 and Supplemental Table 2) [162]. This result, along with the polyclonal equine hyperimmune serum data in the cynomolgus macaque model, dampened enthusiasm for antibody-based therapy for ebolavirus.

The next attempt to rectify the use of antibody approaches for treatment of filovirus infections came in 2012. Dye et al. used purified polyclonal IgG from EBOV or MARV vaccinated nonhuman primates [163]. Treatment delivered on days 2, 4, and 8 post-homologous EBOV and MARV challenge resulted in survival of all treated animals. This result was striking as the purified anti-EBOV IgG was less potent in neutralizing EBOV than was KZ52, which had failed to protect macaques [162] demonstrating that *in vitro* neutralization does not necessarily correlate with *in vivo* protection. Further partial to complete protection in the macaque model was also reported when using cocktails of three mouse mAbs targeting the EBOV GP administered beginning at either 1 or 2 days after homologous challenge (Table 2). One study used a cocktail of three mouse mAbs (1H3, 2G4, 4G7) designated ZMAb, and resulted in 50–100% protection in the EBOV cynomolgus macaque model depending on the dosing schedule [164]. The other study used a cocktail of three mouse-human chimeric mAbs (13C6, 13F6, 6D8), designated MB-003, and demonstrated 67% protection in the EBOV rhesus macaque model (Table 2) [165]. The MB-003 cocktail was advanced by treating rhesus macaques at the time of first clinical signs. Treatment starting at day 4 post EBOV challenge with two subsequent doses on days 7 and 10 resulted in partial protection [166]. A collaborative effort between the inventors of MB-003 and ZMAb resulted in the product ZMapp, a cocktail of the three mAbs 13C6, 2G4 and 4G7 produced in tobacco plants [167]. ZMapp treatment resulted in complete protection of rhesus macaques when initiated as late as 5 days after EBOV challenge, an advanced stage of disease in this model.

Main functional characteristics of EBOV-directed mAbs are presented in Supplemental Table 3. Although the mechanism of protection for ZMapp is not fully understood, Davidson et al. have shown that mAb 13C6 binds to the tip of the GP glycan cap suggesting complement, antibody-dependent cell-mediated cytotoxicity (ADCC), or another Fc-mediated mechanism. Conversely, mAbs 2G4 and 4G7 bind to epitopes in the GP base neutralizing the virus by a structural mechanism [168]. ZMapp has been further optimized into MIL77, a cocktail of two mAbs, 13C6 and 2G4, produced in Chinese hamster ovary cells [169]. Other groups have recently explored human anti-EBOV GP mAbs as treatments in NHPs. Specifically, monotherapy with mAb114 completely protected rhesus monkeys when treated beginning as late as 5 days (treatment regimen on days 5, 6, and 7) after EBOV challenge [170].

During the West African EBOV outbreak, ZMapp, ZMAb, or MIL77 were used compassionately to treat patients repatriated to Europe and the US [151,171–174]. Unfortunately, the role of these mAbs in patient survival is difficult to discern as patients had also received advanced supportive care and most of them an additional experimental therapy. In 2015, the Partnership for Research on ebolavirus in Liberia II (PREVAIL II) performed a randomized controlled trial of ZMapp versus the available standard of care alone in Guinea and Sierra Leone [175]. ZMapp plus standard care was superior, but unfortunately fell short of the prespecified probability threshold of 97.5% superiority to standard care alone in prevention of 28-day mortality, and thus the result lacked statistical significance. In the more recent 2018–2020 DRC EBOV outbreak, a clinical trial (Pamoja Tulinde Maisha; PALM trial) comparing three mAb-based approaches, ZMapp (Mapp Biopharmaceutical, cocktail of three mAbs), REGN-EB3 [Regeneron Pharmaceuticals, cocktail of mAb 3470 (atoltivimab), 3471 (odesivimab) and 3479 (maftivimab)] and mAb114 (Ridgeback Biotherapeutics, single mAb), with remdesivir (Gilead Sciences), a small-molecule antiviral drug (see below), was performed. The PALM trial reported significantly improved efficacy for mAb114 or REGN-EB3 over ZMapp and remdesivir [176]. Interestingly, for unknown reasons, ZMapp performed worse in this trial than in the earlier PREVAIL II trial. Overall, in

Table 3. Monoclonal antibody characteristics.

mAb	Therapeutic Details	Binding site on EBOV GP	Isotype	Production	Characteristics	Virus species targeted	Status
REGN3479 [1,2]	Component of REGN-EB3	Binds at the base of GP, between GP1 and GP2 protomers, parallel with viral surface. Binding may block cathepsin cleavage or release of fusion loop following receptor binding [1]	Human IgG1 [1,3]	Low fucose CHO cells	Produced in VelocImmune mice (encoding human VL and VH gene regions and mouse Fc), immunized with DNA and protein EBOV GP immunogens, then human IgG1 Fc exchanged for mouse Fc [1,3] Neutralizes EBOV GP, does not trigger FcγRIIIa signaling, does not bind sGP [1]	EBOV	REGN-EB3 showed superiority with respect to mortality at interim analysis as 1 of 4 treatments in 2018/2019 clinical efficacy trial (NCT03719586) [4]
REGN3470 [1,2]	Component of REGN-EB3	Binds to outside of glycan cup, perpendicular to GP [1]	As above	Low fucose CHO cells	As above Neutralizes EBOV GP, triggers FcγRIIIa signaling, does not bind sGP [1]	EBOV	As above
REGN3471 [1,2]	Component of REGN-EB3	Binds close to center of GP chalice, perpendicular (90°) to viral surface. [1] Binds closer to center than mAb c13C6, but further away from NPC1 Loop C binding site than mAb 114 [1]	As above	Low fucose CHO cells	As above No neutralizing ability, triggers FcγRIIIa signaling, binds sGP [1]	EBOV	As above
mAb 114 [2,5,6]	Single human mAb therapeutic	Binds glycan cap region (competes with mAb 13C6 binding) [5]	Human IgG1 [2,5]	293 FreeStyle cells (Invitrogen) [5], and CHO cells [2]	Human mAb isolated from EBV-immortalized memory B cell in blood of survivor from 1995 Kikwit (DRC) EVD outbreak 11 years post-infection [5] Neutralizes EBOV GP, mediates Fc-dependent ADCC. In contrast to mAb 13C6, does not require complement for neutralization [5]	EBOV	Showed superiority with respect to mortality at interim analysis as one of 4 treatments in 2018/19 clinical efficacy trial (NCT03719586) [4]
mAb 100 [5]	Not developed as therapeutic	Binds at base of GP (competes with KZ52 binding) [5] Rotated 90° compared to REGN3479 binding [1] Interferes with proteolytic processing and RBS exposure [7]	Human IgG1 [5]	293 FreeStyle (Invitrogen) [5]	As above Neutralizes EBOV GP, mediates Fc-dependent ADCC [5]	EBOV	

Table 3. (Continued).

mAb	Therapeutic Details	Binding site on EBOV GP	Isotype	Production	Characteristics	Virus species targeted	Status
mAb c13C6 [8]	Component of ZMapp™	Binds glycan cap (overlapping c1H3 epitope) oriented perpendicular to viral membrane [8] Competes with c1H3 binding [8]	Mouse/human chimeric IgG1 [9]	*Nicotiana benthamiana* (tobacco plant)-based (afucosylated)	Parental mouse mAb produced by vaccination of mice with Venezuelan equine encephalitis (VEE) replicons expressing EBOV GP [10] EBOV GP Neutralizing but requires complement [9,11] Binds to sGP [10]	EBOV	ZMapp™ showed superiority to standard of care alone in 2014/15 PREVAIL II Phase I/II Safety and Efficacy Study, corresponding to lowering of relative risk of mortality by 40% [2,12] However, ZMapp™ showed to be inferior with respect to mortality at interim analysis as one of 4 treatments in 2018/19 clinical efficacy trial (NCT03719586), and discontinued [4]
mAb c2G4 [8]	Component of ZMapp™	Binds GP base at or near interface of GP1 and GP2 (similar to KZ52), overlapping with binding site of c4G7. Binds at bottom of GP with upward angle toward GP1 [8] Competes with c4G7 binding [8] Interferes with proteolytic processing and RBS exposure [7]	Mouse/human chimeric IgG1 [11]	*Nicotiana benthamiana* (tobacco plant)-based (afucosylated)	Parental mouse mAb produced by vaccination of mice with VSVΔG/ZEBOVGP followed by boost with EBOV virus-like particles (VLPs) [13] Neutralizes EBOV GP [11]	EBOV	As above
mAb c4G7 [8]	Component of ZMapp™	Binds GP base at or near interface of GP1 and GP2 (similar to KZ52), overlapping with binding site of c2G4. Binds perpendicular to GP [8] Competes with c2G4 binding [8] Interferes with proteolytic processing and RBS exposure [7]	Mouse/human chimeric IgG1 [11]	*Nicotiana benthamiana* (tobacco plant)-based (afucosylated)	Parental mouse mAb produced by vaccination of mice with VSVΔG/ZEBOVGP followed by boost with EBOV VLPs [13] Neutralizes EBOV GP [11]	EBOV	As above

Table 3. (Continued).

mAb	Therapeutic Details	Binding site on EBOV GP	Isotype	Production	Characteristics	Virus species targeted	Status
mAb ADI-15 878 [7]	Component of MBP134^{AF}	Binds GP base, and competes with KZ52 binding [14] Discontinuous epitope in both GP1 and GP2 subunits spanning neighboring protomers. Includes putative membrane- seeking residues in GP2 internal fusion loop [7] In contrast to other base binders (mAb 100, KZ52, c2G4 and c4G7), ineffective at blocking GP cleavage, but rather targets and neutralizes cleaved GP species formed deep in endocytic pathway [7]	Human IgG1	FreeStyle 293-F cells	Human mAb from a library of 349 mAbs prepared from B cells from blood of survivor (subject 45) from 2014 EBOV outbreak three months after diagnosis of patient with EBOV infection [14]	All 5 EBOV species [7]	
mAb ADI-23774 [7]	Component of MBP134^{AF}	Binds GP base, and competes with KZ52 binding [14] Does not compete with ADI-15,878 binding [15] Binds cleaved form of GP [15]	Human IgG1	FreeStyle 293-F cells	As above [14] The mAb ADI-23,774 is derived from ADI-15,946[14] that has undergone specificity oligonucleotide-based mutagenesis and specificity-maturation to increase binding to SUDV GP [15]	EBOV, SUDV, and BDBV [15]	
mAb EBOV-520 [16]	Component of two mAb cocktail (EBOV-520 and EBOV-548)	Binds GP base[16]. Competes with c2G4 and c4G7 binding, but in contrast to these mAbs binds with highest affinity to cleaved GP. Binds to a unique conformational epitope spanning GP1 and GP1 near RBS, and inhibits receptor engagement unlike ADI-15,878 and 23,774 potentially by altering RBS conformation [7,16]	Engineered for low Fc binding by fusion to IgG1-LALA Fc [16]	CHO cells [17]	Human mAb from 1 of 2 survivors of either the 2014 EVD epidemic in Nigeria or the 2014 Boende outbreak in the Democratic Republic of the Congo. Collected within a year of infection [16] Neutralizing does not need complement [16]	EBOV, SUDV and BDBV [16]	
EBOV-548 [17]	Component of two mAb cocktail (EBOV-520 and EBOV-548)	Binds glycan cap with similar orientation as mAb c13C6, but also makes contact with interior of chalice [17]	Human IgG1	CHO cells [17]	Human mAb from survivor of 2014 EVD epidemic in Nigeria. Collected within a year of infection [16,17]	EBOV and BDBV neutralization. EBOV, BDBV and SUDV binding [17]	EBOV-548 selected based on capacity to cooperatively enhance EBOV-520 binding to multiple EBOV species GP, and decreased virus mutational escape [17]
mAb ch133 [18]	Not developed as therapeutic	Binds within 521–560aa of GP. GP base overlapping KZ52, c2G4 and c4G7 binding sites [8,18]	Mouse/human chimeric IgG1 [18]	CHO cells [18]	Parental mouse mAb produced by immunization with 293 T cells expressing EBOV GP followed by boost with purified GP [19] Neutralizing and does not bind sGP [20]	EBOV	

Table 3. (Continued).

mAb	Therapeutic Details	Binding site on EBOV GP	Isotype	Production	Characteristics	Virus species targeted	Status
mAb ch226 [18]	Not developed as therapeutic	Binds within 1–232aa of GP. Within base of GP but more membrane distal than mAb ch133, close to glycan cap [18,20,21]	Mouse/human chimeric IgG1 [18]	CHO cells [18]	Parental mouse mAb produced by immunization with 293 T cells expressing EBOV GP followed by boost with purified GP [19] Neutralizing and does not bind sGP [20]	EBOV	
mAb KZ52 [22]	Not developed as therapeutic	Binds at base of GP at a conformational epitope comprised of three discontinuous segments: residues 42–43 at N-terminus of GP1, and 505–514 and 549–556 at N-terminus of GP2 of pre-fusion conformation [23]. Binds perpendicular to GP [8] Interferes with proteolytic processing and RBS exposure [7]	Human IgG1	CHO cells	Human mAb derived from survivor from 1995 Kikwit (DRC) EVD outbreak during same year of infection. Produced using Fab phage display libraries prepared from bone marrow, followed by fusion to human IgG1 [24]	EBOV	Following promising results from guinea pig efficacy studies was discontinued after poor efficacy in macaque model [22,25]
mAb h13F6 [11]	Not developed as therapeutic	Binds linear epitope (aa 405 – 413) within GP1 mucin-like domain [10] Does not compete with c6D8 [8]	Mouse/human chimeric IgG1 [9]	*Nicotiana benthamiana* (tobacco plant)-based (afucosylated)	Parental mouse mAb produced by vaccination of mice with VEE replicons expressing EBOV GP [10] Non-neutralizing [9,10]	EBOV	
mAb c6D8 [11]	Not developed as therapeutic	Binds linear epitope (aa 393–401) within GP1 mucin-like domain [10] Does not compete with h13F6 [8]	Mouse/human chimeric IgG1 [9]	*Nicotiana benthamiana* (tobacco plant)-based (afucosylated)	Parental mouse mAb produced by vaccination of mice with VEE replicons expressing EBOV GP [10] EBOV GP neutralizing, but requires complement [9, 10]	EBOV	
mAb c1H3 [11]	Not developed as therapeutic	Binds glycan cap overlapping c13C6 epitope, but mAb oriented at less steep angle [8] Competes with c13C6 binding [8]	Mouse/human chimeric IgG1 [11]	*Nicotiana benthamiana* (tobacco plant)-based (afucosylated)	Parental mouse mAb produced by vaccination of mice with VSVΔG/ZEBOVGP followed by boost with EBOV VLPs [13] Weakly neutralizing [26] Binds sGP [27]	EBOV	

Table references

1.Pascal KE, Dudgeon D, Trefry JC, Anantpadma M, Sakurai Y, Murin CD, et al. Development of clinical-stage human monoclonal antibodies that treat advanced Ebola virus disease in nonhuman primates. J Infect Dis. 2018;218 (suppl_5):S612-S26. Epub 2018/06/04. doi: 10.1093/infdis/jiy285. PubMed PMID: 29,860,496; PubMed Central PMCID: PMCPMC6249601.

2.WHO. WHO R&D Blueprint – Ad-hoc Expert Consultation on clinical trials for Ebola therapeutics 2018. Available from: https://www.who.int/ebola/drc-2018/summaries-of-evidence-experimental-therapeutics.pdf?ua=1. [Accessed 13 January, 2021]

3.Pascal KE, Coleman CM, Mujica AO, Kamat V, Badithe A, Fairhurst J, et al. Pre- and postexposure efficacy of fully human antibodies against Spike protein in a novel humanized mouse model of MERS-CoV infection. Proc Natl Acad Sci U S A. 2015;112(28):8738–43. Epub 2015/07/01. doi: 10.1073/pnas.1510830112. PubMed PMID: 26,124,093; PubMed Central PMCID: PMCPMC4507189.

4.Mulangu S, Dodd LE, Davey RT, Jr., Tshiani Mbaya O, Proschan M, Mukadi D, et al. A Randomized, controlled trial of Ebola virus disease therapeutics. N Engl J Med. 2019;381(24):2293–303. Epub 2019/11/28. doi: 10.1056/NEJMoa1910993. PubMed PMID: 31,774,950.

5.Corti D, Misasi J, Mulangu S, Stanley DA, Kanekiyo M, Wollen S, et al. Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. Science. 2016;351(6279):1339–42. Epub 2016/02/27. doi: 10.1126/science.aad5224. PubMed PMID: 26,917,593.

6.Gaudinski MR, Coates EE, Novik L, Widge A, Houser KV, Burch E, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of the therapeutic monoclonal antibody mAb114 targeting Ebola virus glycoprotein (VRC 608): an open-label phase 1 study. Lancet. 2019;393(10,174):889–98. Epub 2019/01/29. doi: 10.1016/S0140–6736(19)30,036–4. PubMed PMID: 30,686,586; PubMed Central PMCID: PMCPMC6436835.

7.Wec AZ, Herbert AS, Murin CD, Nyakatura EK, Abelson DM, Fels JM, et al. Antibodies from a human survivor define sites of vulnerability for broad protection against Ebolaviruses. Cell. 2017;169(5):878–90 e15. Epub 2017/05/20. doi: 10.1016/j.cell.2017.04.037. PubMed PMID: 28,525,755; PubMed Central PMCID: PMCPMC5808922.
8.Murin CD, Fusco ML, Bornholdt ZA, Qiu X, Olinger GG, Zeitlin L, et al. Structures of protective antibodies reveal sites of vulnerability on Ebola virus. Proc Natl Acad Sci U S A. 2014;111(48):17,182–7. Epub 2014/11/19. doi: 10.1073/pnas.1414164111. PubMed PMID: 25,404,321; PubMed Central PMCID: PMCPMC4260551.
9.Olinger GG, Jr., Pettitt J, Kim D, Working C, Bohorov O, Bratcher B, et al. Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques. Proc Natl Acad Sci U S A. 2012;109(44):18,030–5. Epub 2012/10/17. doi: 10.1073/pnas.1213709109. PubMed PMID: 23,071,322; PubMed Central PMCID: PMCPMC3497800.
10.Wilson JA, Hevey M, Bakken R, Guest S, Bray M, Schmaljohn AL, et al. Epitopes involved in antibody-mediated protection from Ebola virus. Science. 2000;287(5458):1664–6. Epub 2000/03/04. doi: 10.1126/science.287.5458.1664. PubMed PMID: 10,698,744.
11.Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti JB, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature. 2014;514(7520):47–53. Epub 2014/08/30. doi: 10.1038/nature13777. PubMed PMID: 25,171,469; PubMed Central PMCID: PMCPMC4214273.
12.Group PIW, Multi-National PIIST, Davey RT, Jr., Dodd L, Proschan MA, Neaton J, et al. A Randomized, controlled trial of ZMapp for Ebola virus infection. N Engl J Med. 2016;375(15):1448–56. Epub 2016/10/13. doi: 10.1056/NEJMoa1604330. PubMed PMID: 27,732,819; PubMed Central PMCID: PMCPMC5086427.
13.Qiu X, Fernando L, Melito PL, Audet J, Feldmann H, Kobinger G, et al. Ebola GP-specific monoclonal antibodies protect mice and guinea pigs from lethal Ebola virus infection. PLoS Negl Trop Dis. 2012;6(3):e1575. Epub 2012/03/27. doi: 10.1371/journal.pntd.0001575. PubMed PMID: 22,448,295; PubMed Central PMCID: PMCPMC3308939.
14.Bornholdt ZA, Turner HL, Murin CD, Li W, Sok D, Souders CA, et al. Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak. Science. 2016;351(6277):1078–83. Epub 2016/02/26. doi: 10.1126/science.aad5788. PubMed PMID: 26,912,366; PubMed Central PMCID: PMCPMC4900763.
15.Wec AZ, Bornholdt ZA, He S, Herbert AS, Goodwin E, Wirchnianski AS, et al. Development of a human antibody cocktail that deploys multiple functions to confer pan-ebolavirus protection. Cell Host Microbe. 2019;25(1):39–48 e5. Epub 2019/01/11. doi: 10.1016/j.chom.2018.12.004. PubMed PMID: 30,629,917; PubMed Central PMCID: PMCPMC6396299.
16.Gilchuk P, Kuzmina N, Ilinykh PA, Huang K, Gunn BM, Bryan A, et al. Multifunctional pan-ebolavirus antibody recognizes a site of broad vulnerability on the ebolavirus glycoprotein. Immunity. 2018;49(2):363–74 e10. Epub 2018/07/22. doi: 10.1016/j.immuni.2018.06.018. PubMed PMID: 30,029,854; PubMed Central PMCID: PMCPMC6104738.
17.Gilchuk P, Murin CD, Milligan JC, Cross RW, Mire CE, Ilinykh PA, et al. Analysis of a Therapeutic antibody cocktail reveals determinants for cooperative and broad ebolavirus neutralization. Immunity. 2020;52(2):388–403 e12. Epub 2020/02/06. doi: 10.1016/j.immuni.2020.01.001. PubMed PMID: 32,023,489; PubMed Central PMCID: PMCPMC7111202.
18.Marzi A, Yoshida R, Miyamoto H, Ishijima M, Suzuki Y, Higuchi M, et al. Protective efficacy of neutralizing monoclonal antibodies in a nonhuman primate model of Ebola hemorrhagic fever. PLoS One. 2012;7(4):e36192. Epub 2012/05/05. doi: 10.1371/journal.pone.0036192. PubMed PMID: 22,558,378; PubMed Central PMCID: PMCPMC3338609.
19.Takada A, Watanabe S, Okazaki K, Kida H, Kawaoka Y. Infectivity-enhancing antibodies to Ebola virus glycoprotein. J Virol. 2001;75(5):2324–30. Epub 2001/02/13. doi: 10.1128/JVI.75.5.2324–2330.2001. PubMed PMID: 11,160,735; PubMed Central PMCID: PMCPMC114815.
20.Takada A, Feldmann H, Stroeher U, Bray M, Watanabe S, Ito H, et al. Identification of protective epitopes on ebola virus glycoprotein at the single amino acid level by using recombinant vesicular stomatitis viruses. J Virol. 2003;77(2):1069–74. Epub 2002/12/28. doi: 10.1128/jvi.77.2.1069–1074.2003. PubMed PMID: 12,502,822; PubMed Central PMCID: PMCPMC140786.
21.Audet J, Wong G, Wang H, Lu G, Gao GF, Kobinger G, et al. Molecular characterization of the monoclonal antibodies composing ZMAb: a protective cocktail against Ebola virus. Sci Rep. 2014;4:6881. Epub 2014/11/07. doi: 10.1038/srep06881. PubMed PMID: 25,375,093; PubMed Central PMCID: PMCPMC5381473.
22.Oswald WB, Geisbert TW, Davis KJ, Geisbert JB, Sullivan NJ, Jahrling PB, et al. Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. PLoS Pathog. 2007;3(1):e9. Epub 2007/01/24. doi: 10.1371/journal.ppat.0030009. PubMed PMID: 17,238,286; PubMed Central PMCID: PMCPMC1779296.
23.Lee JE, Fusco ML, Hessell AJ, Oswald WB, Burton DR, Saphire EO. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature. 2008;454(7201):177–82. Epub 2008/07/11. doi: 10.1038/nature07082. PubMed PMID: 18,615,077; PubMed Central PMCID: PMCPMC2700032.
24.Maruyama T, Rodriguez LL, Jahrling PB, Sanchez A, Khan AS, Nichol ST, et al. Ebola virus can be effectively neutralized by antibody produced in natural human infection. J Virol. 1999;73(7):6024–30. Epub 1999/06/11. doi: 10.1128/JVI.73.7.6024–6030.1999. PubMed PMID: 10,364,354; PubMed Central PMCID: PMCPMC112663.
25.Parren PW, Geisbert TW, Maruyama T, Jahrling PB, Burton DR. Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. J Virol. 2002;76(12):6408–12. Epub 2002/05/22. doi: 10.1128/jvi.76.12.6408–6412.2002. PubMed PMID: 12,021,376; PubMed Central PMCID: PMCPMC136210.
26.Qiu X, Audet J, Wong G, Pillet S, Bello A, Cabral T, et al. Successful treatment of ebola virus-infected cynomolgus macaques with monoclonal antibodies. Sci Transl Med. 2012;4(138):138ra81. Epub 2012/06/16. doi: 10.1126/scitranslmed.3003876. PubMed PMID: 22,700,957.
27.Qiu X, Alimonti JB, Melito PL, Fernando L, Stroher U, Jones SM. Characterization of Zaire ebolavirus glycoprotein-specific monoclonal antibodies. Clin Immunol. 2011;141(2):218–27. Epub 2011/09/20. doi: 10.1016/j.clim.2011.08.008. PubMed PMID: 21,925,951.

the PALM trial, antibody specificity, initial higher antibody doses, and perhaps the more favorable pharmacokinetics of human antibodies may have conferred an advantage. Despite the success, a considerable downside of ZMapp, REGN-EB3, and MAb114 is their high specificity for EBOV infections with little to no cross-protective efficacy against other ebolaviruses. More recently, strategically engineered, next-generation human mAbs (i.e. MBP134, FVM04, and CA45) have demonstrated the desired cross-protective efficacy against EBOV, SUDV, and BDBV in preclinical studies and thus are promising new mAb-based approaches for future outbreaks [177,178]. On 14th October 2020, REGN-EB3 (called Inmazeb™, Regeneron) became the first drug approved by the US FDA for treatment of EBOV [179]. Based on the product quality review literature supplied by the US FDA, treatment consists of a single intravenous infusion of the three mAb cocktail comprised 50 mg/kg each of the three mAbs (atoltivimab, odesivimab and maftivimab), and is approved for treatment of EBOV infection in adult and pediatric patients, including neonates born to RT-PCR EBOV-positive mothers [180].

2.3.2.2. Nucleoside analogs. Nucleoside analogs are a group of direct-acting antivirals that have also shown efficacy against EBOV in the stringent NHP model. For those tested in controlled human clinical trials, these drugs have shown differing levels of efficacy.

2.3.2.2.1. BCX4430 (Galidesivir). BCX4430, developed by BioCryst Pharmaceuticals, was the first small-molecule inhibitor to show protective capacity against filovirus infection, in this case MARV, in a NHP model [181]. Consistent with the frequent repurposing of direct-acting antiviral drugs within a class to target alternative viruses, BCX4430 was originally developed as an inhibitor of hepatitis C virus (HCV) [182]. BCX4430, also has the advantage of being administered via the IM route, and hence would be more suitable for use in the low healthcare infrastructure EBOV treatment setting than drugs administered IV.

Structurally, BCX4430 is an adenosine ribose analog that was designed as a non-obligate chain terminator (see below). Similar to most nucleoside-based inhibitors, the BCX4430 parent compound is activated by intracellular kinases into the active triphosphate form (BCX4430-TP) after being taken up by cells. This form is incorporated by the viral RdRp enzyme into the nascent viral RNA strand followed by its inhibition of the RdRp RNA synthesis function [181]. Nucleoside inhibitors are classified into 3 distinct types ('obligate' and 'non-obligate' chain terminators and RNA 'mutators'), which is based on their mode of RdRp inhibition mechanism and is dictated by molecular structure of the drug [183]. BCX4430 is a non-obligate chain terminator as the molecule contains a 3′-OH group which enables RNA chain elongation to occur beyond its site of incorporation. After incorporation of an additional one or two residues the RdRp is inhibited [181], presumably by a BCX4430-induced alteration in the conformation of the nascent RNA molecule, although the precise mechanistic details remain unclear.

After showing initial high levels of efficacy against multiple filoviruses in rodent models, BCX4430 was shown to provide protection in NHPs (cynomolgus macaques) from lethal MARV infection when treatment was initiated up to 48 hours post-infection, with an associated decrease in virus serum RNA levels [181]. In addition to potent, low micromolar inhibition of filoviruses, BCX4430 showed moderate levels of *in vitro* inhibitory activity against members of other virus families associated with human disease [181]. Peer-reviewed follow-up studies in the EBOV NHP model have yet to be published. Meeting abstracts and press-releases have reported differing levels of efficacy against EBOV disease [182,184], and a Phase 1 trial studying safety, tolerability, and pharmacokinetics of IV administered BCX4430 was recently completed [185].

2.3.2.2.2. GS-5734 (Remdesivir). Similar to BCX4430, GS-5734 is an adenosine ribose-based non-obligate chain terminator that targets the viral RdRp. GS-5734 is the prodrug form of GS-441524 a drug that was original discovered as a broad range inhibitor of multiple RNA viruses and the HCV RdRp through *in vitro* screening of a series of 1′-substituted analogs of 4-aza-7,9-dideazaadenosine, a nucleoside analog with potent cytotoxicity in cancer cell lines [186], by Gilead Sciences [187]. By modification of GS-441524, GS-5734 enabled bypass of the initial phosphorylation step, which is rate limiting for production of the active tri-phosphorylated form of the drug [188]. Consistent with higher intracellular concentrations of the active triphosphate form resulting from its altered metabolism, GS-5734 was shown to have higher potency than GS-441524 against multiple viruses [188,189]. GS-441524 and GS-5734 activity against EBOV was initially reported in an HTS screen of ~1000 nucleoside and phosphonate analogues [190].

Testing of GS-5734 in established EBOV rodent models is generally prevented by the short half-life of the drug in these species due to the presence of high levels of a secreted carboxylesterase 1c (*Ces1c*) that is absent in primates [188,191,192]. Although BCX4430 was the first small-molecule inhibitor to show efficacy against filoviruses in NHPs, GS-5734 was the first to show efficacy specifically against EBOV in NHPs, which is regarded as a virus more difficult to control than MARV in these animals [188]. Following IV administration, the highest dosing of GS-5734 provided protection from lethal infection when administered starting 72 hours post-infection, with viral RNA levels being reduced to below the detection limit in the majority of animals [188]. GS-5734 was shown to have potent *in vitro* inhibitory activity against MARV as well as other ebolavirus species in a variety of different cell types [188]. The accumulation of the active metabolite within multiple sites including the testes and eyes, suggested that the drug may also prove useful to eradicate the virus from 'sanctuary' sites of EBOV persistence [188].

Expanding on earlier studies [187], GS-5734 was shown to have *in vitro* inhibitory activity against a broad range of RNA viruses [188,193], which recently translated into *in vivo* efficacy against Nipah, MERS and SARS-CoV-2 in NHPs [189,194,195]. Following compassionate use in two EVD patients [172,190], both of which survived, a clinical trial for EVD patients in the DRC was initiated (PALM trial, see above), based in large part on the NHP efficacy data [188]. This randomized clinical trial was comprised of a direct side-by-side comparison of GS-5734 with three different mAb-based therapeutics in 681 EVD patients during the DRC EBOV outbreak [176]. Patients were enrolled after an average of 5.5 days following onset of

symptoms. Interim analysis resulted in termination of the GS-5734 arm based on inferiority, with an overall increase in 28-day mortality rate of approximately 20% regardless of stratification for disease severity based on viremia compared to the best performing mAb therapy (see above) [176]. The results of this study presumably reflect the greater potency and half-life of fully humanized mAbs over virus-targeting drugs. However, even the best mAb cocktail was still associated with an overall mortality of over 60%. This indicates that there is still more to do regarding use of therapeutics for patient disease management, possibly involving use of mixtures of mAbs and drugs such as GS-5734, perhaps in combination with drugs that ameliorate the over-reactive response of the host associated with infection. It also emphasizes the need to detect and treat infected patients as early as possible.

2.3.2.2.3. T-705 (favipiravir). T-705 is a fluorinated derivative (six-member aromatic ring containing 2 N atoms) of a pyrazine compound originally discovered through a HTS screen of a chemical library for inhibitory drugs against influenza A by Toyama Chemical Co. Ltd [196,197]. Similar to other nucleoside analogues, the tri-phosphorylated form of T-705 is the active molecule, which has been suggested to function as a purine analog. The modes of action are believed to be both through RNA mutagenesis, serving to increase the error rate resulting in genomic catastrophe, as well as by causing premature chain termination [197,198]. The drug was shown to have potent inhibitory activity against multiple influenza A, B and C viruses, which translated to efficacy in preclinical animal models. Clinical trials showed efficacy against seasonal influenza A, and the drug was licensed for use against novel or reemerging influenza A in Japan in 2014 [198]. T-705 is also available for oral administration, which may have advantages in resource-poor settings.

Similar to BCX4430 and GS-5734, T705 has been shown to have inhibitory effect against a broad range of RNA viruses [197], with a moderate level of potency against EBOV *in vitro* [199]. Efficacy studies in rodent models showed substantial levels of protection even when drug treatment was delayed until 6 days after EBOV infection (Supplemental Table 1) [199]. Although an antiviral effect was seen in terms of a moderate (2 to 3-log) reduction in viremia and increase in time to death, translation into NHPs was associated with only low to moderate levels of protection, even when treatment was initiated prior to EBOV infection (Table 1) [200–202]. The decreased viremia was dose dependent and associated with an increase in mutations consistent with T-705 mode of action as an RNA mutagen [201].

Based in part on the preclinical efficacy rodent studies, an open multicenter nonrandomized clinical trial (called the JIKI trial) was conducted, which with 126 patients was the largest therapeutic trial conducted during the 2013–2015 EBOV epidemic [202]. Patients, when stratified based on disease severity, showed no significant beneficial effect of T-705, although in patients with less severe disease a trend toward better survival was observed. Subsequent analysis suggests that one reason for the poor response may have been the failure to reach desired plasma trough levels due to unexpected pharmacokinetics of T-705 [202] Together with the inferiority of GS-5734 in the PALM trial, this suggests that RdRp targeting drugs may, by themselves not be sufficient to control EBOV when administered later in the course of disease following symptom onset. However, the capacity for oral dosing of T-705 does raise the possibility for use in a high-risk exposure setting, which may not be suited to drugs and mAb-based therapeutics that are given via parenteral routes. Given the recent commercial approval of a highly efficacious EBOV vaccine, such high-risk exposure may have most utility for outbreaks involving other ebolavirus species and related filoviruses.

2.3.2.2.4. CMX001 (Brincidofovir). Although DNA rather than RNA viruses, the herpesvirus family has been the focus of intensive nucleoside analogue inhibitor development over the past three decades. Cidofovir (CDV), a deoxycytidine analogue, and its more bioavailable lipid-conjugated orally administered prodrug form, CMX001 (Brincidofovir), function as non-obligate chain terminators [203]. CMX001 was developed by Chimerix Inc [204] to overcome certain limitations of CDV in response to an identified need by the US Department of Health and Humans Services for a medical countermeasure to treat symptomatic smallpox disease in a growing smallpox naïve world. CMX001, which was created by conjugation of a lipid moiety to the CDV molecule, has a number of advantages over its CDV parent molecule in level of cellular uptake, oral bioavailability, and decreased nephrotoxicity [204,205].

CMX001 was shown to potently inhibit EBOV and other ebolavirus species in multiple cell types *in vitro* [206]. Interestingly, the lipid side chain of CMX001 rather than the CDV-PP molecule appeared critical for activity against EBOV, with CDV itself being shown to have poor activity against EBOV at the levels used for analysis (<50μM) [206]. During the 2013–2015 epidemic, the US FDA authorized CMX001 for compassionate use in EVD patients, and a single-arm Phase 2 trial was initiated in Liberia (NCT02271347) [207]. Results from mouse studies not available at the time subsequently showed lack of efficacy against EBOV [206], and differences in pharmacokinetics of the drug prevented testing in NHPs [204,207]. Following treatment of a total of four patients the manufacturer, Chimerix Inc, terminated the trial. No patient completed the treatment course and none survived to day 14, the period of assessment for the primary survival outcome. Consistent with earlier safety data, no adverse or serious adverse reactions were identified [207].

2.3.2.3. Small interfering RNAs (siRNA). Early approaches to target the viral replication machinery involved the use of small interfering RNAs (siRNAs) to achieve degradation of viral mRNAs (TKM-Ebola) [208] or phosphorodiamidate morpholino oligomer (PMO) to block translation of viral proteins [209]. TKM-Ebola was developed to target VP35, VP24 and L and chemically modified to avoid induction of a nonspecific innate immune response and packaged in novel lipid nanoparticle (LNP) for delivery. IV treatment of rhesus macaques starting 30 minutes after EBOV challenge and again at either days 1, 3, and 5 or daily through day 6 after virus exposure resulted in 66% and 100% protection, respectively [208]. The PMO-based approaches were most successful in animal models, including NHPs, when targeting VP24 (AVI-6002 and AVI-7537) [209].

A few years later, TKM-Ebola was modified by eliminating the anti-VP24 siRNA and modifying the LNP (TKM-100802).

During the 2013–2015 West African EBOV outbreak, TKM-100802 was further modified to ensure specificity to the EBOV Makona strain by substitutions in the VP35 and L siRNAs (TKM-130803) [210]. This formulation was again modified by changing the LNP to an LNP2 composition. Evaluation of the new TKM-130803 in rhesus macaques challenged with the EBOV Makona strain and treated IV (0.5 mg/kg) beginning on day 3 after challenge for a total of seven daily treatments resulted in complete protection of the animals [211].

During the West Africa EBOV outbreak, TKM-100802 was administered to five patients and to one individual as postexposure prophylaxis. Unfortunately, efficacy and safety could not be assessed as the individuals were also treated with other products [212]. Subsequently, the newer TKM-130803 formulation was assessed in a small single-arm phase 2 trial conducted in Sierra Leone [210]. Infected patients received single daily IV administrations for 7 days resulting in three survivors out of 12 treated patients. TKM-130803 appeared safe in this poorly conducted trial but showed no clear efficacy. The siRNA technology has also been applied to filoviruses other than EBOV in pre-clinical NHP models [213,214]. The siRNA technology was not considered as part of the DRC PALM trial.

3. Conclusions

The filovirus field has come a long way over more than three decades in finding prophylactic and therapeutic countermeasures for EBOV infections as reviewed in this article. Many modalities, identified through *in vitro* screens or repurposing strategies, had been confirmed and characterized in preclinical animal work over the years, but clinical trials remained rather limited. The devastating West African (2013–2015) and recent second largest EBOV outbreak in the northeastern DRC (2018–2020) became game changing events for the translation of countermeasures into clinical trials ultimately resulting in licensure of several EBOV vaccines, but only a single EBOV treatment modality utilizing a mAb cocktail (Inmazeb™, Regeneron).

In general, therapeutic interventions have lagged behind vaccine development. State-of-the-art supportive care is most critical for the success of EVD patient management and should be the foundation for any treatment strategy. Currently, the mAb-based approaches targeting EBOV GP demonstrate the highest level of efficacy in clinical trials (PALM trial) and would be the first treatment choice. It is to be expected that more mAb products will be licensed over the next few years. The main disadvantages of mAbs are high virus specificity, vulnerability to virus escape and evolutionary changes, mAb production cost and time, and repetitive intravenous administration which is less ideal in resource-poor settings.

Direct-acting antivirals are the second treatment choice and likely ideal for combination therapy. For relapsed or persistently infected convalescent patients these drugs may be the only choice. Remdesivir (Veklury®, Gilead) is the leading candidate and was part of the PALM trial, but other nucleoside analogs and distinct polymerase inhibitors should also be continued. The main advantages of this class of drugs are broad spectrum efficacy and lower production cost and time. Future efforts should study synergistic and/or additive effects of these compounds in combination with mAbs.

Looking forward, future treatment development should focus on broad spectrum direct-acting compounds (i.e. mAbs and nucleoside analogs) with efficacy after a single dose that can be adminstered via less invasive oral and intranasal routes. The treatment modalities need also to be affordable and meet logistical constraints for provision to those most in need living in resource-poor settings.

4. Expert opinion

Due to their high potency, long half-life and low-toxicity mAb-based therapeutics are superior in performance, especially in the symptomatic period, to any nucleoside analog – the only other therapeutic class that has consistently shown efficacy against EBOV in NHP models. Nucleoside analogs may have a more potent affect when used in combination therapy, and orally administered nucleoside analogs may play an important role in protection against high-risk exposure prior to or at very early stages of infection. Notably, regardless of demonstrated *in vitro* inhibitory activity and efficacy in rodent models, host-directed monotherapy therapeutics consistently fail in more stringent preclinical models.

The mAb cocktail, Inmazeb™ (Regeneron), became the first drug approved by the US FDA for treatment of EBOV infection [179] (approved 14th October 2020). However, what we have learned for EBOV will undoubtedly be applicable to manage related filovirus outbreaks, with more recent work being focused increasingly on development of mAbs and RdRp-targeting therapeutics with broader range that encompass these related viruses. Through the optics of the present COVID-19 pandemic, preemptive development and manufacture of efficacious, yet inexpensive broadly acting therapeutics validated in the most stringent preclinical models available *through to the point of licensure* is critical if we are to avoid further reactive and largely ineffective responses to future filovirus outbreaks. These drugs should be suitable for use in multiple scenarios, from high-risk exposure to severely diseased patients.

Given the relative high level of success of mAb therapy for treatment of EVD, development of technology for more rapid generation and production of virus-specific mAbs is expected to remain an area of focus. While mAbs show clear superiority, at least in the symptomatic patient, cost makes them prohibitively expensive, preventing widespread use – especially in the countries in which filoviruses normally emerge. Development of alternative strategies that take advantage of mAb technology, but with a focus to cost reduction whether through more effective routes of administration (IM and mucosal delivery) or ease and efficiency of manufacture should be an area of increased focus to broaden application of this promising therapeutic intervention.

An area that warrants further attention is combination therapy, with drugs ideally targeting different individual aspects of the filovirus lifecycle susceptible to therapeutic control. A number of studies have identified combinations of host-directed drugs that functioned synergistically against EBOV *in vitro* [123,215]. An obvious choice is the combination of mAbs, which function at the level of entry, with direct-acting antivirals

such as GS-5734 that inhibit at the level of the RdRp. This approach may also address the issue of virus persistence, by potentially preventing the establishment of persistent infection within immune privileged sites. Therapies and combinations more amenable to use following high-risk exposure, such as those able to rapidly achieve necessary systemic levels following oral administration, should also be developed, particularly as at this early stage of infection, the virus may be more susceptible to pharmacologic control.

Most of the achievements in treatment of filovirus diseases have been targeted to EBOV. Future efforts need to be focused on more broadly acting therapies whether it be mAbs targeting multiple ebolaviruses or direct-acting antivirals targeting RdRp or a combination of both. Drug screening programs will remain as useful tools in therapeutic development strategies. However, the past has shown that *in vitro* efficacy is largely insufficient for selecting a candidate. Small rodent models such as the mouse are a necessary confirmatory step even though they are often still not sufficiently predictive. The guinea pig and ultimately NHP model are key components of preclinical screening for filovirus intervention programs. We advise future reports on antiviral therapeutics against filoviruses to include a minimal *in vivo* pre-clinical component to help to limit costs and time in the drug development process.

Over the next years, the field needs to prioritize refinement of current promising approaches and move them through clinical trials for licensure application. Those drugs (or combinations thereof) then need to be produced to sufficient quantities and properly stored for immediate and *uncomplicated* release and administration. With lower priority, second-generation drug development programs should continue as needed and funding allows. If the COVID-19 pandemic has taught us anything, it is that preemption is by far less costly in lives and resources than reaction with poor preparation. Finally, early and rapid diagnosis in combination with immediate isolation of cases and thorough contact tracing cannot be replaced by any therapeutic intervention. These public health measures are a necessary prerequisite for any successful therapeutic intervention strategy in future filovirus outbreaks.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. International Committee on Taxonomy of Viruses Executive C. The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks. Nat Microbiol. 2020 May;5(5):668–674.
2. Kuhn JH, Amarasinghe GK, Basler CF, et al. Filoviridae. 2019 [cited; Available from: https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna-viruses/mononegavirales/w/filoviridae
3. Goldstein T, Anthony SJ, Gbakima A, et al. The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. Nat Microbiol. 2018 Oct;3(10):1084–1089.
4. Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. Trends Microbiol. 2007 Sep;15(9):408–416.
5. Olivero J, Fa JE, Real R, et al. Recent loss of closed forests is associated with Ebola virus disease outbreaks. Sci Rep. 2017 Oct 30;7(1):14291.
6. Wolfe ND, Daszak P, Kilpatrick AM, et al. Bushmeat hunting, deforestation, and prediction of zoonoses emergence. Emerg Infect Dis. 2005 Dec;11(12):1822–1827.
7. WHO. Ebola in the Democratic Republic of the Congo North Kivu, Ituri 2018–2020. 2020 [cited; Available from: https://www.who.int/emergencies/diseases/ebola/drc-2019
8. Centers for Disease Control and Prevention NCfEaZIDN, Division of High-Consequence Pathogens and Pathology (DHCPP), Viral Special Pathogens Branch (VSPB). 2020 Democratic Republic of the Congo, equateur province. 2020 Nov 18 [cited; Available from: https://www.cdc.gov/vhf/ebola/outbreaks/drc/2020-june.html [Accessed 13 January, 2021]
9. Lo TQ, Marston BJ, Dahl BA, et al. Ebola: anatomy of an epidemic. Annu Rev Med. 2017 Jan;14(68):359–370.
10. De Cock KM, El-Sadr WM. A tale of two viruses: HIV, Ebola and health systems. AIDS. 2015 Jun 1;29(9):989–991.
11. Wells CR, Pandey A, Ndeffo Mbah ML, et al. The exacerbation of ebola outbreaks by conflict in the democratic republic of the congo. Proc Natl Acad Sci USA. 2019 Nov 26;116(48):24366–24372.
12. Strindberg S, Maisels F, Williamson EA, et al. Guns, germs, and trees determine density and distribution of gorillas and chimpanzees in Western Equatorial Africa. Sci Adv. 2018 Apr;4(4):eaar2964.
13. Formenty P, Hatz C, Le Guenno B, et al. Human infection due to Ebola virus, subtype cote d'Ivoire: clinical and biologic presentation. J Infect Dis. 1999 Feb;179(Suppl 1):S48–53.
14. Wang LF, Anderson DE. Viruses in bats and potential spillover to animals and humans. Curr Opin Virol. 2019 Feb;34:79–89.
15. Hayman DT, Yu M, Crameri G, et al. Ebola virus antibodies in fruit bats, Ghana, West Africa. Emerg Infect Dis. 2012 Jul;18(7):1207–1209.
16. Ogawa H, Miyamoto H, Nakayama E, et al. Seroepidemiological prevalence of multiple species of filoviruses in fruit bats (eidolon helvum) migrating in Africa. J Infect Dis. 2015 Oct 1;212(Suppl 2):S101–8.
17. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. Nature. 2005 Dec 1;438(7068):575–576.
18. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020 Feb 22;395(10224):565–574.
19. Mari Saez A, Weiss S, Nowak K, et al. Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol Med. 2015 Jan;7(1):17–23.

20. Geisbert TW, Young HA, Jahrling PB, et al. Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. J Infect Dis. 2003 Dec 1;188(11):1618–1629.
21. Baseler L, Chertow DS, Johnson KM, et al. The pathogenesis of Ebola virus disease. Annu Rev Pathol. 2017 Jan;24(12):387–418.
22. Geisbert TW, Hensley LE, Larsen T, et al. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. Am J Pathol. 2003 Dec;163(6):2347–2370.
23. Bosio CM, Aman MJ, Grogan C, et al. Ebola and Marburg viruses replicate in monocyte-derived dendritic cells without inducing the production of cytokines and full maturation. J Infect Dis. 2003 Dec 1;188(11):1630–1638.
24. Wauquier N, Becquart P, Padilla C, et al. Human fatal Zaire ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. PLoS Negl Trop Dis. 2010 Oct 5;4(10):e837.
25. Mahanty S, Hutchinson K, Agarwal S, et al. Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses. J Immunol. 2003 Mar 15;170(6):2797–2801.
26. Geisbert TW, Young HA, Jahrling PB, et al. Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. Am J Pathol. 2003 Dec;163(6):2371–2382.
27. Geisbert TW, Hensley LE, Gibb TR, et al. Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. Lab Invest. 2000 Feb;80(2):171–186.
28. Gupta M, Spiropoulou C, Rollin PE. Ebola virus infection of human PBMCs causes massive death of macrophages, CD4 and CD8 T cell sub-populations in vitro. Virology. 2007 Jul 20;364(1):45–54.
29. Hensley LE, Young HA, Jahrling PB, et al. Proinflammatory response during Ebola virus infection of primate models: possible involvement of the tumor necrosis factor receptor superfamily. Immunol Lett. 2002 Mar 1;80(3):169–179.
30. Mate SE, Kugelman JR, Nyenswah TG, Ladner JT, Wiley MR, Cordier-Lassalle T, et al. Molecular evidence of sexual transmission of Ebola virus. N Engl J Med. 2015 Dec 17;373(25): 2448–2454.
31. Hunt CL, Lennemann NJ, Maury W. Filovirus entry: a novelty in the viral fusion world. Viruses. 2012 Feb;4(2):258–275.
32. Moller-Tank S, Maury W. Ebola virus entry: a curious and complex series of events. PLoS Pathog. 2015 Apr;11(4):e1004731.
33. Rhein BA, Maury WJ. Ebola virus entry into host cells: identifying therapeutic strategies. Curr Clin Microbiol Rep. 2015 Sep;2(3):115–124.
34. Herbert AS, Davidson C, Kuehne AI, et al. Niemann-pick C1 is essential for ebolavirus replication and pathogenesis in vivo. mBio. 2015 May 26;6(3):e00565–15.
35. Bhattacharyya S, Mulherkar N, Chandran K. Endocytic pathways involved in filovirus entry: advances, implications and future directions. Viruses. 2012 Dec;4(12):3647–3664.
36. Miller EH, Chandran K. Filovirus entry into cells - new insights. Curr Opin Virol. 2012 Apr;2(2):206–214.
37. Dahlmann F, Biedenkopf N, Babler A, et al. Analysis of Ebola virus entry into macrophages. J Infect Dis. 2015 Oct 1;212(Suppl 2):S247–57.
38. Hoenen T, Groseth A, Feldmann H. Therapeutic strategies to target the Ebola virus life cycle. Nat Rev Microbiol. 2019 Oct;17(10):593–606.
39. Sanchez A, Geisbert TW, Feldmann H. Filoviridae: marburg and Ebola viruses. In: Knipe DM, Howley PM, editors. Fields virology. 5th ed ed. Lippincott Williams & Wilkins; 2013. p. 1409–1448.
40. Muhlberger E, Weik M, Volchkov VE, et al. Comparison of the transcription and replication strategies of Marburg virus and Ebola virus by using artificial replication systems. J Virol. 1999 Mar;73(3):2333–2342.
41. Hoenen T, Shabman RS, Groseth A, et al. Inclusion bodies are a site of ebolavirus replication. J Virol. 2012 Nov;86(21):11779–11788.
42. Mehedi M, Falzarano D, Seebach J, et al. A new Ebola virus nonstructural glycoprotein expressed through RNA editing. J Virol. 2011 Jun;85(11):5406–5414.
43. Takahashi K, Halfmann P, Oyama M, et al. DNA topoisomerase 1 facilitates the transcription and replication of the Ebola virus genome. J Virol. 2013 Aug;87(16):8862–8869.
44. Fang J, Pietzsch C, Ramanathan P, et al. Staufen1 interacts with multiple components of the Ebola virus ribonucleoprotein and enhances viral RNA synthesis. mBio. 2018 Oct 9;9(5):5.
45. Martin S, Chiramel AI, Schmidt ML, et al. A genome-wide siRNA screen identifies a druggable host pathway essential for the Ebola virus life cycle. Genome Med. 2018 Aug 7;10(1):58.
46. Biedenkopf N, Hartlieb B, Hoenen T, et al. Phosphorylation of Ebola virus VP30 influences the composition of the viral nucleocapsid complex: impact on viral transcription and replication. J Biol Chem. 2013 Apr 19;288(16):11165–11174.
47. Modrof J, Muhlberger E, Klenk HD, et al. Phosphorylation of VP30 impairs ebola virus transcription. J Biol Chem. 2002 Sep 6;277(36):33099–33104.
48. Kruse T, Biedenkopf N, Hertz EPT, et al. The Ebola virus nucleoprotein recruits the host PP2A-B56 phosphatase to activate transcriptional support activity of VP30. Mol Cell. 2018 Jan 4;69(1):136–45 e6.
49. Banadyga L, Hoenen T, Ambroggio X, et al. Ebola virus VP24 interacts with NP to facilitate nucleocapsid assembly and genome packaging. Sci Rep. 2017 Aug 9;7(1):7698.
50. Watt A, Moukambi F, Banadyga L, et al. A novel life cycle modeling system for Ebola virus shows a genome length-dependent role of VP24 in virus infectivity. J Virol. 2014 Sep;88(18):10511–10524.
51. Schudt G, Dolnik O, Kolesnikova L, et al. Transport of Ebolavirus nucleocapsids is dependent on actin polymerization: live-cell imaging analysis of ebolavirus-infected cells. J Infect Dis. 2015 Oct 1;212(Suppl 2):S160–6.
52. Takamatsu Y, Kolesnikova L, Becker S. Ebola virus proteins NP, VP35, and VP24 are essential and sufficient to mediate nucleocapsid transport. Proc Natl Acad Sci U S A. 2018 Jan 30;115(5):1075–1080.
53. Adu-Gyamfi E, Digman MA, Gratton E, et al. Single-particle tracking demonstrates that actin coordinates the movement of the Ebola virus matrix protein. Biophys J. 2012 Nov 7;103(9):L41–3.
54. Han Z, Harty RN. Packaging of actin into Ebola virus VLPs. Virol J. 2005 Dec;20(2):92.
55. Noda T, Ebihara H, Muramoto Y, et al. Assembly and budding of ebolavirus. PLoS Pathog. 2006 Sep;2(9):e99.
56. Ruthel G, Demmin GL, Kallstrom G, et al. Association of ebola virus matrix protein VP40 with microtubules. J Virol. 2005 Apr;79(8):4709–4719.
57. Feldmann H, Nichol ST, Klenk HD, et al. Characterization of filoviruses based on differences in structure and antigenicity of the virion glycoprotein. Virology. 1994 Mar;199(2):469–473.
58. Volchkov VE, Feldmann H, Volchkova VA, et al. Processing of the Ebola virus glycoprotein by the proprotein convertase furin. Proc Natl Acad Sci U S A. 1998 May 12;95(10):5762–5767.
59. Martin-Serrano J, Zang T, Bieniasz PD. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. Nat Med. 2001 Dec;7(12):1313–1319.
60. Licata JM, Simpson-Holley M, Wright NT, et al. Overlapping motifs (PTAP and PPEY) within the Ebola virus VP40 protein function independently as late budding domains: involvement of host proteins TSG101 and VPS-4. J Virol. 2003 Feb;77(3):1812–1819.
61. Han Z, Sagum CA, Bedford MT, et al. ITCH E3 Ubiquitin ligase interacts with Ebola virus VP40 to regulate budding. J Virol. 2016 Oct 15;90(20):9163–9171.
62. Harty RN, Brown ME, Wang G, et al. A PPxY motif within the VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: implications for filovirus budding. Proc Natl Acad Sci U S A. 2000 Dec 5;97(25):13871–13876.
63. Nanbo A, Maruyama J, Imai M, et al. Ebola virus requires a host scramblase for externalization of phosphatidylserine on the surface of viral particles. PLoS Pathog. 2018 Jan;14(1):e1006848.
64. Snoy PJ. Establishing efficacy of human products using animals: the US food and drug administration's "animal rule". Vet Pathol. 2010 Sep;47(5):774–778.
65. Yamaoka S, Banadyga L, Bray M, et al. Small animal models for studying filovirus pathogenesis. Curr Top Microbiol Immunol. 2017;411:195–227.

66. Geisbert TW, Strong JE, Feldmann H. Considerations in the use of nonhuman primate models of Ebola virus and Marburg virus infection. J Infect Dis. 2015 Oct 1;212(Suppl 2):S91–7.
67. Reynolds P, Ebola MA. Marburg virus vaccines. Virus Genes. 2017 Aug;53(4):501–515.
68. Feldmann H, Feldmann F, Ebola: MA. Lessons on vaccine development. Annu Rev Microbiol. 2018 Sep;8(72):423–446.
69. Mirza MU, Vanmeert M, Ali A, et al. Perspectives towards antiviral drug discovery against Ebola virus. J Med Virol. 2019 Dec;91 (12):2029–2048.
70. Humeniuk R, Mathias A, Cao H, et al. Safety, tolerability, and pharmacokinetics of remdesivir, an antiviral for treatment of COVID-19, in Healthy Subjects. Clin Transl Sci. 2020 Sep;13(5):896–906.
71. Bluth J BioCryst Completes Phase 1 Clinical Trial of Galidesivir. 2019.
72. Bixler SL, Bocan TM, Wells J, et al. Intracellular conversion and in vivo dose response of favipiravir (T-705) in rodents infected with Ebola virus. Antiviral Res. 2018 Mar;151:50–54.
73. Ronfeld RA, Tremaine LM, Wilner KD. Pharmacokinetics of sertraline and its N-demethyl metabolite in elderly and young male and female volunteers. Clin Pharmacokinet. 1997;32(Suppl 1):22–30.
74. WHO. WHO R&D blueprint – ad-hoc expert consultation on clinical trials for Ebola therapeutics 2018 [cited; Available from: https://www.who.int/ebola/drc-2018/summaries-of-evidence-experimental-therapeutics.pdf?ua=1
75. Pascal KE, Dudgeon D, Trefry JC, et al. Development of clinical-stage human monoclonal antibodies that treat advanced Ebola virus disease in nonhuman primates. J Infect Dis. 2018 Nov 22;218(suppl_5):S612–S26.
76. Gaudinski MR, Coates EE, Novik L, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of the therapeutic monoclonal antibody mAb114 targeting Ebola virus glycoprotein (VRC 608): an open-label phase 1 study. Lancet. 2019 Mar 2;393(10174):889–898.
77. Qiu X, Fernando L, Melito PL, et al. Ebola GP-specific monoclonal antibodies protect mice and guinea pigs from lethal Ebola virus infection. PLoS Negl Trop Dis. 2012;6(3):e1575.
78. Audet J, Wong G, Wang H, et al. Molecular characterization of the monoclonal antibodies composing ZMAb: a protective cocktail against Ebola virus. Sci Rep. 2014 Nov;6(4):6881.
79. Cross RW, Bornholdt ZA, Prasad AN, et al. Prior vaccination with rVSV-ZEBOV does not interfere with but improves efficacy of postexposure antibody treatment. Nat Commun. 2020 Jul 27;11(1):3736.
80. WHO. Categorization and prioritization of drugs for consideration for testing or use in patients infected with Ebola. 2015 [cited; Available from: https://www.who.int/medicines/ebola-treatment/2015_0703TablesofEbolaDrugs.pdf?ua=1
81. Wec AZ, Bornholdt ZA, He S, et al. Development of a human antibody cocktail that deploys multiple functions to confer pan-Ebolavirus protection. Cell Host Microbe. 2019 Jan 9;25(1):39–48 e5.
82. Gilchuk P, Murin CD, Milligan JC, et al. Analysis of a therapeutic antibody cocktail reveals determinants for cooperative and broad Ebolavirus neutralization. Immunity. 2020 Feb 18;52(2):388–403 e12.
83. Kuzmina NA, Younan P, Gilchuk P, et al. Antibody-dependent enhancement of Ebola virus infection by human antibodies isolated from survivors. Cell Rep. 2018 Aug 14;24(7):1802–15 e5.
84. Maruyama T, Rodriguez LL, Jahrling PB, et al. Ebola virus can be effectively neutralized by antibody produced in natural human infection. J Virol. 1999 Jul;73(7):6024–6030.
85. Takada A, Ebihara H, Jones S, et al. Protective efficacy of neutralizing antibodies against Ebola virus infection. Vaccine. 2007 Jan 22;25(6):993–999.
86. Marzi A, Yoshida R, Miyamoto H, et al. Protective efficacy of neutralizing monoclonal antibodies in a nonhuman primate model of Ebola hemorrhagic fever. PLoS One. 2012;7(4):e36192.
87. DiMasi JA, Grabowski HG, Hansen RW. Innovation in the pharmaceutical industry: new estimates of R&D costs. J Health Econ. 2016 May;47:20–33.
88. Zhao Z, Martin C, Fan R, et al. Drug repurposing to target Ebola virus replication and virulence using structural systems pharmacology. BMC Bioinformatics. 2016 Feb;18(17):90.
89. Azvolinsky A. Repurposing existing drugs for new indications. The Scientist. 2016;30(3):36–41.
90. Mercorelli B, Palu G, Loregian A. Drug repurposing for viral infectious diseases: how far are we? Trends Microbiol. 2018 Oct;26 (10):865–876.
91. Adalja A, Broad-spectrum antiviral IT. Agents: a crucial pandemic tool. Expert Rev Anti Infect Ther. 2019 Jul;17(7):467–470.
92. Jarvis MA, Hansen FA, Rosenke K, et al. Antivir ther. In: Evaluation of drugs for potential repurposing against COVID-19 using a tier-based scoring system. Aug, 2020. 3.
93. Postnikova E, Cong Y, DeWald LE, et al. Testing therapeutics in cell-based assays: factors that influence the apparent potency of drugs. PLoS One. 2018;13(3):e0194880.
94. Emeny JM, Morgan MJ. Regulation of the interferon system: evidence that Vero cells have a genetic defect in interferon production. J Gen Virol. 1979 Apr;43(1):247–252.
95. Chew T, Noyce R, Collins SE, et al. Characterization of the interferon regulatory factor 3-mediated antiviral response in a cell line deficient for IFN production. Mol Immunol. 2009 Jan;46 (3):393–399.
96. Schnittler HJ, Feldmann H. Viral hemorrhagic fever–a vascular disease? Thromb Haemost. 2003 Jun;89(6):967–972.
97. Martines RB, Ng DL, Greer PW, et al. Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses. J Pathol. 2015 Jan;235(2):153–174.
98. Zivcec M, Spiropoulou CF, Spengler JR. The use of mice lacking type I or both type I and type II interferon responses in research on hemorrhagic fever viruses. Part 2: vaccine efficacy studies. Antiviral Res. 2020 Feb;174:104702.
99. Sweiti H, Ekwunife O, Jaschinski T, et al. Repurposed therapeutic agents targeting the ebola virus: a systematic review. Curr Ther Res Clin Exp. 2017;84:10–21.
100. Bixler SL, Duplantier AJ, Bavari S. Discovering drugs for the treatment of Ebola virus. Curr Treat Options Infect Dis. 2017;9(3):299–317.
101. Schuler J, Hudson ML, Schwartz D, et al. Review of computational drug discovery, development, and repurposing for Ebola virus disease treatment. Molecules. 2017 Oct 20;22(10):1777.
102. Wang X, Zou P, Wu F, et al. Development of small-molecule viral inhibitors targeting various stages of the life cycle of emerging and re-emerging viruses. Front Med. 2017 Dec;11(4):449–461.
103. Hernandez HW, Soeung M, Zorn KM, et al. High throughput and computational repurposing for neglected diseases. Pharm Res. 2018 Dec 17;36(2):27.
104. Edwards MR, Basler CF. Current status of small molecule drug development for Ebola virus and other filoviruses. Curr Opin Virol. 2019 Apr;35:42–56.
105. Fanunza E, Frau A, Corona A, et al. Antiviral agents against Ebola virus infection: repositioning old drugs and finding novel small molecules. Annu Rep Med Chem. 2018;51:135–173.
106. Pecheur EI, Borisevich V, Halfmann P, et al. The synthetic antiviral drug Arbidol inhibits globally prevalent pathogenic viruses. J Virol. 2016 Jan 6;90(6):3086–3092.
107. Zhao Y, Ren J, Harlos K, et al. Toremifene interacts with and destabilizes the Ebola virus glycoprotein. Nature. 2016 Jul 7;535 (7610):169–172.
108. Nelson EA, Barnes AB, Wiehle RD, et al. Clomiphene and its isomers block ebola virus particle entry and infection with similar potency: potential therapeutic implications. Viruses. 2016 Aug 2;8(8):206.
109. Fan H, Du X, Zhang J, et al. Selective inhibition of Ebola entry with selective estrogen receptor modulators by disrupting the endolysosomal calcium. Sci Rep. 2017 Jan;24(7):41226.
110. Li H, Yu F, Xia S, et al. Chemically modified human serum albumin potently blocks entry of ebola pseudoviruses and viruslike particles. Antimicrob Agents Chemother. 2017;61(4):Apr.
111. Nelson EA, Dyall J, Hoenen T, et al. The phosphatidylinositol-3-phosphate 5-kinase inhibitor apilimod blocks filoviral entry and infection. PLoS Negl Trop Dis. 2017 Apr;11(4):e0005540.
112. Ren J, Zhao Y, Fry EE, Stuart DI. Target Identification and. Mode of action of four chemically divergent drugs against Ebolavirus infection. J Med Chem. 2018 Feb 8;61(3): 724–733.
113. Lindstrom A, Anantpadma M, Baker L, et al. Phenotypic prioritization of diphyllin derivatives that block filoviral cell entry by

vacuolar (h(+))-atpase inhibition. ChemMedChem. 2018 Dec 20;13 (24):2664–2676.
114. Sakurai Y, Sakakibara N, Toyama M, et al. Novel amodiaquine derivatives potently inhibit Ebola virus infection. Antiviral Res. 2018 Dec;160:175–182.
115. Hulseberg CE, Feneant L, Szymanska-de Wijs KM, et al. Arbidol and other low-molecular-weight drugs that inhibit Lassa and Ebola viruses. J Virol. 2019 Apr 15;93(8):8.
116. Coves-Datson EM, Dyall J, DeWald LE, King SR, Dube D, Legendre M, et al. Inhibition of Ebola virus by a molecularly engineered banana lectin. PLoS Negl Trop Dis. 2019 Jul;13(7):e0007595.
117. Johansen LM, Brannan JM, Delos SE, et al. FDA-approved selective estrogen receptor modulators inhibit Ebola virus infection. Sci Transl Med. 2013 Jun 19;5(190):190ra79.
118. Dowall SD, Bewley K, Watson RJ, et al.Antiviral screening of multiple compounds against Ebola virus. Viruses. 2016 Oct 27;Vol. 8:11.
119. Johansen LM, DeWald LE, Shoemaker CJ, et al. A screen of approved drugs and molecular probes identifies therapeutics with anti-Ebola virus activity. Sci Transl Med. 2015 Jun 3;7(290):290ra89.
120. Madrid PB, Chopra S, Manger ID, et al. A systematic screen of FDA-approved drugs for inhibitors of biological threat agents. PLoS One. 2013;8(4):e60579.
121. Wang Y, Cui R, Li G, et al. Teicoplanin inhibits Ebola pseudovirus infection in cell culture. Antiviral Res. 2016 Jan;125:1–7.
122. Anantpadma M, Kouznetsova J, Wang H, et al. Large-scale screening and identification of novel Ebola Virus and marburg virus entry inhibitors. Antimicrob Agents Chemother. 2016 Aug;60(8):4471–4481.
123. Dyall J, Nelson EA, DeWald LE, et al. Identification of combinations of approved drugs with synergistic activity against Ebola virus in cell cultures. J Infect Dis. 2018 Nov 22;218(suppl_5):S672–S78.
124. Lane T, Anantpadma M, Freundlich JS, et al. the natural product eugenol is an inhibitor of the Ebola virus in vitro. Pharm Res. 2019 May 17;36(7):104.
125. Towner JS, Paragas J, Dover JE, et al. Generation of eGFP expressing recombinant Zaire ebolavirus for analysis of early pathogenesis events and high-throughput antiviral drug screening. Virology. 2005 Feb 5;332(1):20–27.
126. Madrid PB, Panchal RG, Warren TK, et al. Evaluation of Ebola virus inhibitors for drug repurposing. ACS Infect Dis. 2015 Jul 10;1 (7):317–326.
127. Anantpadma M, Lane T, Zorn KM, et al. Ebola virus Bayesian machine learning models enable new in vitro leads. ACS Omega. 2019 Jan 31;4(1):2353–2361.
128. Mirza MU, Ikram N. Integrated computational approach for virtual hit identification against Ebola viral proteins VP35 and VP40. Int J Mol Sci. 2016 Oct 26;17(11):1748.
129. Chopra G, Kaushik S, Elkin PL, et al. Combating ebola with repurposed therapeutics using the CANDO platform. Molecules. 2016 Nov 25;21(12):12.
130. Penny CJ, Vassileva K, Jha A, et al. Mining of Ebola virus entry inhibitors identifies approved drugs as two-pore channel pore blockers. Biochim Biophys Acta Mol Cell Res. 2019 Jul;1866 (7):1151–1161.
131. Ekins S, Freundlich JS, Clark AM, et al. Machine learning models identify molecules active against the Ebola virus in vitro. F1000Res. 2015;4:1091.
132. Lane TR, Massey C, Comer JE, et al. Repurposing the antimalarial pyronaridine tetraphosphate to protect against Ebola virus infection. PLoS Negl Trop Dis. 2019 Nov;13(11):e0007890.
133. Ekins S, Lingerfelt MA, Comer JE, et al. Efficacy of tilorone dihydrochloride against Ebola virus infection. Antimicrob Agents Chemother. 2018 Feb;62(2):2.
134. Lane TR, Comer JE, Freiberg AN, et al. Repurposing quinacrine against Ebola virus infection in vivo. Antimicrob Agents Chemother. 2019 Sep;63(9). DOI:10.1128/AAC.01142-19.
135. Uebelhoer LS, Albarino CG, McMullan LK, et al. High-throughput, luciferase-based reverse genetics systems for identifying inhibitors of Marburg and Ebola viruses. Antiviral Res. 2014 Jun;106:86–94.
136. Welch SR, Guerrero LW, Chakrabarti AK, et al. Lassa and Ebola virus inhibitors identified using minigenome and recombinant virus reporter systems. Antiviral Res. 2016 Dec;136:9–18.
137. Kouznetsova J, Sun W, Martinez-Romero C, et al. Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs. Emerg Microbes Infect. 2014 Dec;3(12):e84.
138. Xiao JH, Rijal P, Schimanski L, et al. Characterization of influenza virus pseudotyped with ebolavirus glycoprotein. J Virol. 2018 Feb 15;92(4). DOI:10.1128/JVI.00941-17.
139. Salata C, Calistri A, Parolin C, et al. Antiviral activity of cationic amphiphilic drugs. Expert Rev Anti Infect Ther. 2017 May;15 (5):483–492.
140. Dowall SD, Bosworth A, Watson R, et al. Chloroquine inhibited Ebola virus replication in vitro but failed to protect against infection and disease in the in vivo guinea pig model. J Gen Virol. 2015 Dec;96(12):3484–3492.
141. Honko AN, Johnson JC, Marchand JS, et al. High dose sertraline monotherapy fails to protect rhesus macaques from lethal challenge with Ebola virus Makona. Sci Rep. 2017 Jul 19;7(1):5886.
  - **Demonstrated the lack of translation for host-directed agents with high efficacy in *in vitro* and rodent models to efficacy in NHPs.**
142. Mendoza EJ, Qiu X, Kobinger GP. Progression of Ebola therapeutics during the 2014–2015 outbreak. Trends Mol Med. 2016 Feb;22 (2):164–173.
143. Turone F. Doctors trial amiodarone for Ebola in Sierra Leone. Br Med J. 2014;349(nov27 1):g7198.
144. Gupta-Wright A. Concerns about the off-licence use of amiodarone for Ebola. Br Med J. 2015;350(jan20 17):h272.
145. National Institutes of Health UNLoM. Clinicaltrials.gov. 2020 [cited; Available from: https://clinicaltrials.gov
146. Emond RT, Evans B, Bowen ET, et al. A case of Ebola virus infection. Br Med J. 1977 Aug 27;2(6086):541–544.
147. Mupapa K, Massamba M, Kibadi K, et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International scientific and technical committee. J Infect Dis. 1999 Feb;179(Suppl 1):S18–23.
148. Jahrling PB, Geisbert JB, Swearengen JR, et al. Ebola hemorrhagic fever: evaluation of passive immunotherapy in nonhuman primates. J Infect Dis. 2007 Nov 15;196(Suppl 2):S400–3.
149. Mire CE, Geisbert JB, Agans KN, et al. Passive immunotherapy: assessment of convalescent serum against Ebola virus Makona infection in nonhuman primates. J Infect Dis. 2016 Oct 15;214(suppl 3):S367–S74.
150. van Griensven J, Edwards T, de Lamballerie X, et al. Evaluation of convalescent plasma for Ebola virus disease in Guinea. N Engl J Med. 2016 Jan 7;374(1):33–42.
151. Lyon GM, Mehta AK, Varkey JB, et al. Clinical care of two patients with Ebola virus disease in the United States. N Engl J Med. 2014 Dec 18;371(25):2402–2409.
152. Florescu DF, Kalil AC, Hewlett AL, et al. Administration of brincidofovir and convalescent plasma in a patient with Ebola virus disease. Clin Infect Dis. 2015 Sep 15;61(6):969–973.
153. Mora-Rillo M, Arsuaga M, Ramirez-Olivencia G, et al. Acute respiratory distress syndrome after convalescent plasma use: treatment of a patient with Ebola virus disease contracted in Madrid, Spain. Lancet Respir Med. 2015 Jul;3(7):554–562.
154. Kraft CS, Hewlett AL, Koepsell S, et al. The use of TKM-100802 and convalescent plasma in 2 patients with Ebola virus disease in the United States. Clin Infect Dis. 2015 Aug 15;61(4):496–502.
155. Kudoyarova-Zubavichene NM, Sergeyev NN, Chepurnov AA, et al. Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. J Infect Dis. 1999 Feb;179(Suppl 1):S218–23.
156. Jahrling PB, Geisbert TW, Geisbert JB, et al. Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. J Infect Dis. 1999 Feb;179 (Suppl 1):S224–34.
157. Jahrling PB, Geisbert J, Swearengen JR, et al. Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses.. Arch Virol Suppl. 1996;11:135–140.
158. Wilson JA, Hevey M, Bakken R, et al. Epitopes involved in antibody-mediated protection from Ebola virus. Science. 2000 Mar 3;287 (5458):1664–1666.

159. Zucca E. Therapeutic use of monoclonal antibodies: a new era? Ann Oncol. 1998 Jul;9(7):683–685.
160. Drewe E, Powell RJ. Clinically useful monoclonal antibodies in treatment. J Clin Pathol. 2002 Feb;55(2):81–85.
161. Parren PW, Geisbert TW, Maruyama T, et al. Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. J Virol. 2002 Jun;76 (12):6408–6412.
162. Oswald WB, Geisbert TW, Davis KJ, et al. Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. PLoS Pathog. 2007 Jan;3(1):e9.
163. Dye JM, Herbert AS, Kuehne AI, et al. Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. Proc Natl Acad Sci USA. 2012 Mar 27;109(13):5034–5039.
   - **First demonstration for anti-EBOV antibodies providing protection against lethal challenge in NHPs.**
164. Qiu X, Audet J, Wong G, et al. Successful treatment of ebola virus-infected cynomolgus macaques with monoclonal antibodies. Sci Transl Med. 2012 Jun 13;4(138):138ra81.
165. Olinger GG, Jr., Pettitt J, Kim D, Working C, Bohorov O, Bratcher B, et al. Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques. Proc Natl Acad Sci U S A. 2012 Oct 30;109(44): 18030–18035.
166. Pettitt J, Zeitlin L, Kim DH, Working C, Johnson JC, Bohorov O, et al. Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. Sci Transl Med. 2013 Aug 21;5(199):199ra13.
167. Qiu X, Wong G, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature. 2014 Oct 2;514 (7520):47–53.
168. Davidson E, Bryan C, Fong RH, et al. Mechanism of binding to Ebola virus glycoprotein by the ZMapp, ZMAb, and MB-003 Cocktail Antibodies. J Virol. 2015 Nov;89(21):10982–10992.
169. Qiu X, Audet J, Lv M, et al. Two-mAb cocktail protects macaques against the Makona variant of Ebola virus. Sci Transl Med. 2016 Mar 9;8(329):329ra33.
170. Corti D, Misasi J, Mulangu S, et al. Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. Science. 2016 Mar 18;351(6279):1339–1342.
171. Pooley W. Ebola: perspectives from a nurse and patient. Am J Trop Med Hyg. 2015 Feb;92(2):223–224.
172. Jacobs M, Rodger A, Bell DJ, et al. Late Ebola virus relapse causing meningoencephalitis: a case report. Lancet. 2016 Jul 30;388 (10043):498–503.
173. Uyeki TM, Mehta AK, Davey RT Jr., et al. Clinical management of Ebola virus disease in the United States and Europe. N Engl J Med. 2016 Feb 18;374(7):636–646.
174. Schibler M, Vetter P, Cherpillod P, et al. Clinical features and viral kinetics in a rapidly cured patient with Ebola virus disease: a case report. Lancet Infect Dis. 2015 Sep;15(9):1034–1040.
175. Group PIW, Multi-National PIIST, Davey RT, Jr., Dodd L, Proschan MA, Neaton J, et al. A Randomized, controlled trial of ZMapp for Ebola virus infection. N Engl J Med. 2016 Oct 13; 375(15):1448–1456.
   - **First controlled clinical trial during 2013–2015 West African epidemic showing superiority of MAb-based (ZMapp) treatment to standard of care.**
176. Mulangu S, Dodd LE, Davey RT Jr., et al. A Randomized, controlled trial of Ebola virus disease therapeutics. N Engl J Med. 2019 Dec 12;381(24):2293–2303.
   - •• **Controlled clinical trial during 2018–2020 DRC outbreak showing superiority of MAbs against EBOV over small molecule inhibitors leading to FDA approval of the first MAb-based treatment for EVD (Inmazeb™).**
177. Bornholdt ZA, Herbert AS, Mire CE, et al. A two-antibody pan-ebolavirus cocktail confers broad therapeutic protection in ferrets and nonhuman primates. Cell Host Microbe. 2019 Jan 9;25(1):49–58 e5.
178. Brannan JM, He S, Howell KA, et al. Post-exposure immunotherapy for two ebolaviruses and Marburg virus in nonhuman primates. Nat Commun. 2019 Jan 10;10(1):105.
179. FDA U. FDA Approves First Treatment for Ebola Virus. 2020 [cited; Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-ebola-virus
180. FDA U. First approval for indication/breakthrough review/priority review. Inmazeb (atoltivimab, maftivimab, odesivimab-ebgn); 2020 30th September 2020.
181. Warren TK, Wells J, Panchal RG, et al. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature. 2014 Apr 17;508(7496):402–405.
182. Madelain V, Nguyen TH, Olivo A, et al. Ebola virus infection: review of the pharmacokinetic and pharmacodynamic properties of drugs considered for testing in human efficacy trials. Clin Pharmacokinet. 2016 Aug;55(8):907–923.
183. Deval J. Antimicrobial strategies: inhibition of viral polymerases by 3′-hydroxyl nucleosides. Drugs. 2009;69(2):151–166.
184. Warren TK, MacLennan S, Mathis A, et al. Efficacy of Galidesivir against Ebola virus disease in rhesus monkeys. [Abstract] open forum of infectious disease. Vol. 4. 2017. p. S55.
185. Pharmaceuticals B BioCryst Completes Phase 1 Clinical Trial of Galidesivir. 2019.
186. Patil SA, Otter BA, Klein RS. 4-Aza-7,9-Dideazaadenosine, a new cytotoxic synthetic c-nucleoside analogue of adenosine. Tetrahedron Lett. 1994;35(30):5339–5342.
187. Cho A, Saunders OL, Butler T, et al. Synthesis and antiviral activity of a series of 1′-substituted 4-aza-7,9-dideazaadenosine C-nucleosides. Bioorg Med Chem Lett. 2012 Apr 15;22(8):2705–2707.
188. Warren TK, Jordan R, Lo MK, et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. Nature. 2016 Mar 17;531(7594):381–385.
   - •• **First demonstration of high level efficacy for a small-molecule direct antiviral as a therapeutic against EBOV in NHPs.**
189. Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med. 2019 May 29;11(494):eaau9242.
190. Siegel D, Hui HC, Doerffler E, et al. Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo[2,1-f][triazin-4-amino] Adenine C-nucleoside (gs-5734) for the treatment of Ebola and emerging viruses. J Med Chem. 2017 Mar 9;60(5):1648–1661.
191. Li B, Sedlacek M, Manoharan I, et al. Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. Biochem Pharmacol. 2005 Nov 25;70(11):1673–1684.
192. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396):eaal3653.
193. Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep. 2017 Mar 6;7(1):43395.
194. de Wit E, Feldmann F, Cronin J, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. Proc Natl Acad Sci USA. 2020 Mar 24;117 (12):6771–6776.
195. Williamson BN, Feldmann F, Schwarz B, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature. 2020 Sep;585(7824):273–276.
196. Furuta Y, Takahashi K, Fukuda Y, et al. In vitro and in vivo activities of anti-influenza virus compound T-705. Antimicrob Agents Chemother. 2002 Apr;46(4):977–981.
197. Furuta Y, Gowen BB, Takahashi K, et al. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res. 2013 Nov;100(2):446–454.
198. Shiraki K, Daikoku T. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. Pharmacol Ther. 2020 May;209:107512.
199. Oestereich L, Ludtke A, Wurr S, et al. Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. Antiviral Res. 2014 May;105:17–21.
200. Bixler SL, Bocan TM, Wells J, et al. Efficacy of favipiravir (T-705) in nonhuman primates infected with Ebola virus or Marburg virus. Antiviral Res. 2018 Mar;151:97–104.
201. Guedj J, Piorkowski G, Jacquot F, et al. Antiviral efficacy of favipiravir against Ebola virus: a translational study in cynomolgus macaques. PLoS Med. 2018 Mar;15(3):e1002535.

202. Madelain V, Mentre F, Baize S, et al. Modeling favipiravir antiviral efficacy against emerging viruses: from animal studies to clinical trials. CPT Pharmacometrics Syst Pharmacol. 2020 May;9(5):258–271.
203. Xiong X, Smith JL, Chen MS. Effect of incorporation of cidofovir into DNA by human cytomegalovirus DNA polymerase on DNA elongation. Antimicrob Agents Chemother. 1997 Mar;41(3):594–599.
204. Lanier R, Trost L, Tippin T, et al. Development of CMX001 for the treatment of poxvirus infections. Viruses. 2010 Dec;2(12):2740–2762.
205. Painter W, Robertson A, Trost LC, et al. First pharmacokinetic and safety study in humans of the novel lipid antiviral conjugate CMX001, a broad-spectrum oral drug active against double-stranded DNA viruses. Antimicrob Agents Chemother. 2012 May;56(5):2726–2734.
206. McMullan LK, Flint M, Dyall J, et al. The lipid moiety of brincidofovir is required for in vitro antiviral activity against Ebola virus. Antiviral Res. 2016 Jan;125:71–78.
207. Dunning J, Kennedy SB, Antierens A, et al. Experimental treatment of Ebola virus disease with brincidofovir. PLoS One. 2016;11(9):e0162199.
208. Geisbert TW, Lee AC, Robbins M, et al. Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study. Lancet. 2010 May 29;375(9729):1896–1905.
209. Iversen PL, Warren TK, Wells JB, et al. Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections. Viruses. 2012 Nov 6;4(11):2806–2830.
210. Dunning J, Sahr F, Rojek A, et al. Experimental treatment of Ebola virus disease with TKM-130803: a single-arm phase 2 clinical trial. PLoS Med. 2016 Apr;13(4):e1001997.
211. Thi EP, Mire CE, Lee AC, et al. Lipid nanoparticle siRNA treatment of Ebola-virus-Makona-infected nonhuman primates. Nature. 2015 May 21;521(7552):362–365.
212. Cross RW, Mire CE, Feldmann H, et al. Post-exposure treatments for Ebola and Marburg virus infections. Nat Rev Drug Discov. 2018 Jun;17(6):413–434.
213. Thi EP, Mire CE, Ursic-Bedoya R, et al. Marburg virus infection in nonhuman primates: therapeutic treatment by lipid-encapsulated siRNA. Sci Transl Med. 2014 Aug 20;6(250):250ra116.
214. Thi EP, Lee AC, Geisbert JB, et al. Rescue of non-human primates from advanced Sudan ebolavirus infection with lipid encapsulated siRNA. Nat Microbiol. 2016 Aug 22;1(10):16142.
215. Sun W, He S, Martinez-Romero C, et al. Synergistic drug combination effectively blocks Ebola virus infection. Antiviral Res. 2017 Jan;137:165–172.