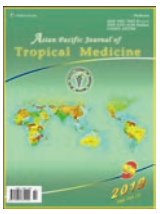

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Ebola virus disease: Recent advances in diagnostics and therapeutics

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ABSTRACT

Ebola virus disease (EVD) is associated with haemorrhagic fever in humans and nonhuman primates, with a high rate of fatality (up to 90%). Some outbreaks in human history have proven the lethality of EVD. The recent epidemic of 2014 and 2015 in West Africa was the deadliest of all time (11 284 deaths). To understand the transmission dynamics, we have reviewed the epidemiology of EVD to date. The absence of any licensed vaccines or approved drugs against Ebola virus (EBOV) further highlights the severity and crisis level of EVD. Some organizations (public and private) are making considerable efforts to develop novel therapeutic approaches or vaccines to contain the outbreak of EBOV shortly. Here, we summarized the various potential drugs and vaccines (undergoing multiple phases of clinical trials) that have arisen as an alternative against EBOV, and we highlighted the numerous issues and limitations hindering this process. Alternatively, an increasing focus on strengthening the medical and civic health structure could provide speedy benefits in containing the spread of EVD, as well as offer a resilient foundation for the deployment of novel drugs and vaccines to the affected countries, once such drugs and vaccines become available.

1. Introduction

Ebola is a lethal disease caused by infection with a virus associated with the Family Filoviridae and the genus *Ebola*. Ebola is also referred to as Ebola haemorrhagic fever[1]. The term 'Ebola' came from the river Ebola, in Sudan and Zaire (Democratic Republic of the Congo), sites where one of the first documented outbreaks of the disease occurred and where the family Filoviridae was discovered. So far, five strains have been identified and four of them have been identified in Africa, including the Zaire Ebola virus (ZEBOV), the Sudan virus (SEBOV), the Taï Forest virus (Taï Forest EBOV, formerly Côte d'Ivoire ebolavirus, and the Bundibugyo virus). All of these strains can make people sick[2]. The fifth, the Reston virus, originated in the Philippines and can cause disease in nonhuman primates (monkeys, gorillas, and chimpanzees[1,3]. As per WHO, Ebola virus disease (EVD) is considered as a fifth-category notifiable transmissible disease due to its severity and lethalness.

After entering the body, Ebola starts killing the cells by making some of them to explode. It weakens and affects the immune system, causing massive bleeding, both internally and externally; and harms practically every organ while diminishing the capacity of the liver and kidneys to function[4]. Signs and symptoms

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typically get initiated in between two days to three weeks after exposure to the deadly virus. Early clinical self-reported symptoms include fever (73%), abdominal pain (60%), muscular pain (76%), headaches (95%) asthenia (86%), myalgia (76%), dysphagia (71%), anorexia (71%), nausea/vomiting (73%), and diarrhoea (73%). Often, fluid loss through vomiting and diarrhoea leads to low blood pressure, followed by rashes[5,6]. Moreover, fewer than ten viral particles are sufficient for distinct contamination. Human-to-human virus transmission is acquired by contact with blood, body fluids (including but not restricted to sweat, drool, faeces, bile, breast milk, urine and organs of an infected person or other animal and contaminated objects (like needles and syringes) of a person suffering with or has died from Ebola[7].

Moreover, male survivors may have the potency to transmit the disease through semen for nearly two months by having oral, vaginal, or anal sex[8]. The possibility of virus transmission continues in dead bodies, and corpses provide a high risk of transmission. Therefore, they must be handled by high-risk contagion-control procedures. From previous studies, it appears that mosquitoes or other insects are not involved in virus transmission, and even spread via air has not been witnessed in the natural habitat[3]. Fruit bats, belonging to Pteropodidae Family, including *Myonycteris torquata*, *Hypsignathus monstrosus*, and *Epomops franquetiare*, are assumed to be the normal carrier of Ebola viruses in nature and can spread the virus without being affected[5].

Additionally, bats are known to carry filoviruses and possess EBOV RNA and antibodies[9]. However, humans and other mammals serve as accidental hosts. In recent decades, exposure to this zoonotic virus has been regarded as a reason for the diminishing numbers of African chimpanzees and gorillas[10].

By evidence and previous statements associated with a similar virus, infection in humans in Guinea and its neighbouring countries was due to contact with the bats or an alive or lifeless animal that has been infected by bats during hunting and meat consumption practices[11]. Indeed, the wild reservoir of this virus is still mysterious and unidentified[9].

2. Time to time EVD outbreak throughout the world

In 1976, the first Ebola outbreak devastated Yambuku, a small village of Zaire, and this was followed by another outbreak, in Nzara, Sudan. The subtype was Zaire EOBV according to the phylogenetic analysis[12,13]. From 1 September to 24 October 1976, 318 cases of acute haemorrhagic viral were logged in northern Zaire, inside a radius of 70 km from Yambuku[14].

An increased number of cases originated in late November 2007 from the township of Bundibugyo and Kikyo in the Bundibugyo District; these townships had approximately 16 000 and 5 700 inhabitants, respectively[2]. On 29 November 2007, WHO and the Uganda Ministry of Health declared an eruption of new EBOV in the Bundibugyo District. On 20 February 2008, there were 93 assumed and 56 laboratory research confirmed cases (30 of whom were hospitalized) and 37 deaths, yielding a 25% case fatality rate. Since the first encounter of EBOV in 1976, EVD has mostly occurred in sub-Saharan Africa. Taken together, cumulative outbreaks of EBOV have been reported as follows: Sudan (1976, 1979, 2004), Democratic Republic of the Congo (1976, 1977, 1995, 2007, 2008, 2012), Gabon (1994, 1996, 2001, 2002), Uganda (2000, 2007, 2011, 2012), and Republic of the Congo (2001, 2002, 2003, 2005)[15].

Ebola also has spread to other countries, originating first in Guinea and consequently spreading across terrestrial borders to Sierra Leone and Liberia by air (1 voyager) to Nigeria and the USA (1 voyager), and by land to Senegal (1 voyager) and Mali (2 voyagers). Until 2013, countries such as Senegal, Spain, United Kingdom, Russia, Philippines, and Italy had also reported Ebola outbreaks. From 1976 to December 2012, an aggregate of 23 outbursts or isolated cases of Ebola had been documented. During these outbreaks, 2 388 Ebola infection cases, including 1 590 deaths, were recorded[16].

At the end of 2013, EBOV broke out in Guinea around Kissidougou, Guéckédou, and Macenta, and consequently extended to at least five additional West African countries[17]. Data obtained on 24 August 2014 from epidemiological and phylogenetic studies demonstrated that this large EVD outbreak in middle Africa was due to the single introduction of a particular EBOV into humans from an unknown reservoir. Thus, all the following human cases (more than 15 000 cases) were derived from one unnamed variant. WHO announced this outbreak as being the most unusual outbreak in history[16]. Reaching up to 2014, outbreaks of EVD were mainly scattered over the central African region[11].

During August 2014, a synchronized EVD outbreak was confirmed in the Democratic Republic of Congo, and a public health catastrophe of international apprehension was declared. The Ebola epidemic in 2014 was the worst outbreak experienced by the human race, more than all the previous outbreaks combined. In the first week of October, nearly 70 cases of infection and 43 deaths were reported in the Democratic Republic of Congo. According to Centers for Disease Control and Prevention, as of 25 September 2014, approximately 6 263 suspected and confirmed cases and 2 917 assumed case deaths had occurred in all parts of five countries in West Africa, and the confirmed cases continued to rise[18]. In 2014, an epidemic that spread over a region of West Africa, was extended to Europe and the United States of America. At that time, total figures of approximately 25 515 infections were projected from this outbreak alone. Of these estimates, 10 572 deaths, including that of 500 healthcare workers, were estimated[19].

Cumulatively, until 4 February 2015, a total of 22 495 cases (confirmed, probable, and suspected) and 8 981 deaths (fatality rate approximately 40%) were documented in nine countries, including Spain, Guinea, Mali, Liberia, Senegal, Nigeria, Sierra Leone along with United States and United Kingdom[20]. On 29 March 2016, WHO terminated the Public Health Emergency of International

Concern status of the EBOV flare-up in West Africa. Until that time, an aggregate of 28 670 affirmed, likely and suspected cases had been recorded in Guinea, Liberia, and Sierra Leone, with a total of 11 325 deaths. Due to the Zaïre strain of EBOV, 9 cases of EBOV, including three deaths, were reported by 22 April 2017[21]. Recently, on 1 August 2018, a new outbreak of Ebola virus disease was announced in North Kivu Province by the Ministry of Health of the Democratic Republic of the Congo. In October 2018, the WHO report concluded 266 total cases of EBOV and 168 deaths[22].

3. EBOV structure–an overview

EBOV is a genera of single-stranded RNA molecules of negative sense. EBOV have a threadlike appearance, with a diameter of 80 nm, and can mould itself into a U-shape similar to a hairpin, or into 6-shaped or circular forms[23]. The family name 'Filoviridae' originates from the Latin word, filum, meaning 'thread' or 'filament', reflecting the morphology of the virus particles as seen with an electron microscope. The EBOV comprises a single strand of negative-sense RNA genome of approximately 18 959 kb in length. Its genome is transcribed into eight major sub genomic mRNAs that encode seven structural proteins and one non-structural protein. These seven genes are in the order 3' leader, nucleoprotein (NP), virion protein35 (VP35), matrix protein (VP40), glycoprotein, virion protein 24 (VP24), virion protein 30 (VP30), and RNA-dependent RNA polymerase (L)-5' tail (Figure 1)[4]. The EBOV RNA genome is encapsulated by a ribonucleoprotein complex of three essential proteins: VP24, VP35 and NP[24]. This complex is involved in transcription and replication, whereas VP30, a minor nucleoprotein, acts as a viral transcriptional activator[25]. VP40 and VP24 are found in a space associated with the nucleocapsid surface for the morphogenesis and budding process of nucleocapsid particle[26]. The trimeric transmembrane glycoprotein (GP) spike, each approximately 7-10 nm long and spaced at approximately 10 nm intervals, are presented on the surface of the virion and are accountable for cellular attachment and entry. Ebo-GP, with a molecular mass of approximately 140 kDa, also encodes host-cell-secreted soluble GP (sGP) and small soluble GP. Two domain receptor-binding subunits, GP1 and the membrane fusion subunit GP2[27], are the cleaved result of the glycoprotein precursor (GP0) by furin enzymes constitute a functional GP tripolymer spike[28]. Recently, researchers have reported the three-dimensional (3D) structure and molecular activities GP spikes within the virion envelop based on X-ray analysis, cryoelectron microscopy (cryoEM), cryoelectron tomography (cryoET), NMR spectroscopy and general virology. The 3D structure, with an achieved resolution of 11 Å, represents the placement of mucin-like domain according to the structure of GP1-GP2 and transmembrane domains. Deniel *et al*. showed that the mucin-like domain blocks the receptor binding sites by covering the glycan cap. Thus, cleavage of the mucin-like domain in the endosomes is required for proper functioning of the NPC-1 binding site[29]. From another study, cryoEM image analysis demonstrated that most filamentous EBOV with a length of ~1 028 nm comprise the cylindrical helical nucleocapsid (NC); however, a longer virus with multiple NCs concerning length has also been observed[30]. Precise knowledge about the structural facts is vital to understand how the safety of the genome, cell binding, access, and immune evasion are attained in animal filamentous virus and to describe how this unique morphology is necessary for pathogenesis. Moreover, detailed structural information would aid an understanding of the mechanism of viral infection along with the identification of key molecules that could be targeted for therapeutic purposes.

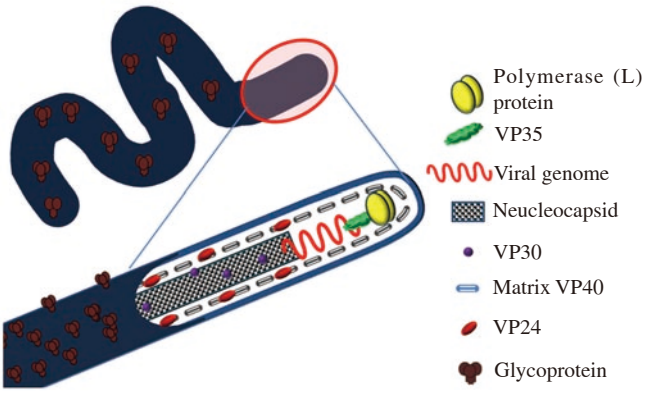

Figure 1. Schematic representation of EBOV ultrastructure indicating its components including viral envelop, matrix, nucleocapsid and various virion proteins.

4. Mechanism of viral entry

To enter host cells, a variety of mechanisms, including clathrin- and caveolae-mediated endocytosis, phagocytosis, and macropinocytosis, has been proposed for EBOV[31]. The mechanism of EBOV cell entry is still poorly understood and has shown different routes of entry in different cell types[32]. Various studies have pronounced different hypotheses; according to some, EBOV internalization depends on low pH and follow the endocytic pathway, whereas several studies have reported that EBOV internalizes through cholesterols, a component of caveolae and lipid-rafts[31,33]. Studies propose that filoviruses entry to the cells from the cell surface is through early endosomes to the late endosomal or lysosomal compartment before the EBOV membranes fuse with cellular membranes to cause viral genome entrance into the cytoplasm (Figure 2)[34]. A complete internalization process through endo/lysosomal compartments has been recently reviewed in[32]. The current report suggests that Human Niemann-Pick C1 (NPC1) cholesterol transporter is essential to host factor during EBOV infection and is predicted to be a polytopic glycoprotein comprising 13 transmembrane helices domains (three large and four small

luminal loops, six small cytoplasmic loops and a cytoplasmic tail)[27,35]. NPC1 resides in the late endoplasmic reticulum and lysosomes of all cells. EBOV enters the host cell through direct binding of the EBOV envelope GP spike to the NPC1 protein. GP acts as a regulator for receptor binding through the mucin domain and membrane fusion mediated by the NPCI protein directly or indirectly, leading to its main aim of virus penetration. The GP from the Filovirus is a type Ⅰ glycoprotein. Both N-linked and O-linked carbohydrates contribute to the noticeably higher molecular weight of the GP compared to the predicted one from deduced amino acid sequences. The additional downstream process behind the GP-NPC1 binding is not clear. However, recent work indicates that a cleaved form of GP1 mediated by host endosomal cysteine proteases is required for binding of GP-NPC1 and induction of GP conformational changes[27]. This alteration of GP arrangement, such as the inclusion of the GP2 hydrophobic fusion loop into the host membrane, and the GP2 unwinding and refolding into a 'six-helix bundle' configuration lead to membrane fusion and release of the EBOV nucleocapsid core into the cytoplasm[36,37]. Another study revealed that EBOV entry is mediated by a membrane-trafficking process, whereby a binding partner for receptor binding region of the EBOV GP is translocated to the cell surface (*e.g.*, lymphocytes and macrophages), and causing cell adhesion of EBOV to the cell surface, followed by internalization[38]. While several studies on the EBOV internalization mechanism into the host cells have advanced our understanding, various key queries remain unanswered and require further scientific approach. For example, further studies are required to analyse the spatial arrangement of membrane spikes, the structural organization of the transmembrane and cytoplasmic proteins, and the spatial interaction between the envelope protein VP40 and the nucleocapsid of EBOV. Analysis of these spatial organizations might help us to understand the vital role of these viral components in the replication cycle of EBOV.

5. Diagnosis of EBOV

Certain analysis of a clinically alleged case of EBOV needs laboratory confirmation (Table 1). EBOV takes up to 3 days after the symptoms to reach a detectable level for proper laboratory evaluation[39]. A diagnostic analysis relying on polymerase chain reaction (PCR) and antigen capture by enzyme-linked immuno sorbent assay (ELISA) and virus isolation kits are available for an early diagnosis of the initial stages of infection[39]. Antibodies such as Immunoglobulin M (Ig M) and Immunoglobulin G (IgG) against the virus can also be marked for diagnosis in the disease progression or later recovery[39,40]. Laboratory outcome in EVD includes thrombocytopenia, leukopenia, and elevated liver enzyme levels. Elevated inflammatory response is noteworthy in patients with asymptomatic EBOV infection. EBOV infection can be easily diagnosed by the prompt action of the immune system[41]. Early and well-regulated inflammatory response with an elevated Interleukin (IL)-6 concentration and IL-1β in a patient is indicative of a good outcome, whereas a wrecked innate immune reaction with excessive activation of macrophage/monocyte with release of IL-10, absence of antibody response, and elevated concentration of IL-1RA, and neopterin after a few days of commencement of disease are associated with a fatal outcome[42]. A simple diagnostic approach to EBOV can be classified into three basic categorizations: 1) serologic tests, 2) antigen test and 3) molecular test. The collection of whole blood, serum or plasma samples of at least 4 mL, with transport to the appropriate health department while the samples are refrigerated or on the ice, is required for further testing. Health departments should be notified immediately after getting positive results. Assay based on Antigen- or antibody or PCR should be executed in level 4 biosafety laboratories because of the extreme biohazard risk. CDC has achieved standardized ELISA for detection of EBOV specific antibodies in infected patients. This test is highly sensitive and can be used for detecting antibodies in humans even ten years after exposure to the EBOV. Drawback associated with current diagnostic analysis is that it takes approximately 2 to 6 hours for analysis, and the expense of around USD 100/sample are hard to meet in resource-constrained West African countries, thus strictly restricting the testing capability. Moreover, time lost without any diagnosis will allow the infected persons to stay in the community and will result in severe risk of unknowingly transferring the virus to others. Hence, the need exists for innovative diagnostics to be developed that eradicate the turnaround time for diagnosis and are cost effective for low-resource countries.

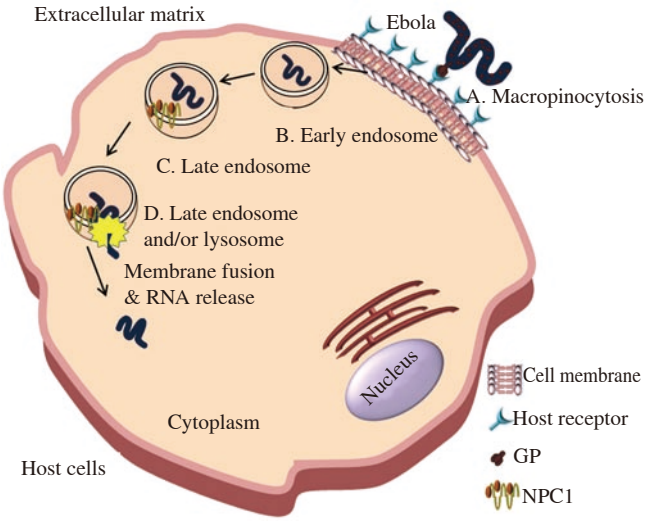

Figure 2. Schematic model of EBOV entry into host cell. (A) GP peplomers of EBOV attaches on to the surface of host the cell membrane *via* receptors. Attachment of GP spikes with receptors triggers macropinocytosis process. (B) EBOV is engulfed by the cells and enclosed in early endosomes. (C) At late endosomal stage, the endosomal cysteine proteases cathepsin B (CatB) and the cathepsin L (CatL) cleaves viral GP for NPC1/GP binding. (D) Late endosome then fuses with lysosomes causing the release of nulceocapsid in to the host cell cytoplasm.

Table 1. Diagnosis of EBOV infection.

Timeline of infection of EBOV	Preferred diagnostic tests
In a few days of initial stage	•Antigen-capture ELISA •IgM ELISA •PCR including real-time quantitate (qPCR) •Virus isolation
Later in infection course or after recovery	•IgM and IgG antibodies
Retrospectively in deceased patients	•Immunohistochemistry analysis •PCR •Virus isolation

6. Existing therapeutic approaches towards EBOV infection

The development of drugs against EBOV has progressed for several decades, but a renewed focus on developing medicines and vaccinations was realized only after the 2014-2016 outbreaks in West Africa, with the concern of potential spread throughout the world. Apart from supportive measures employed during epidemics, such as electrolyte and fluid replacement, oxygen treatment and maintaining acid-base balance in the body, some potential therapeutics were evaluated and tried to control the occurring outbreaks of Ebola.

Drug development requires the identification of probable therapeutic targets that might be a protein, RNA, or any biological component effecting the spread and infective ability of the pathogen. Various strategies such as genetics, biochemical, computational and structural, are generally required to identify the specific therapeutic target either from the host or pathogen. To date, no approved compound/drug is available for the treatment of EVD. However, efforts have been made to screen some candidate drugs for approval, and some of these candidates have been proposed to possess therapeutic potential against EVD. Some such candidates are in different phases of clinical trials and might be a therapeutic answer to EBOV infection.

From a reservoir of approved drugs and mechanistic probes, multiple selective oestrogen receptor modulator (SERM) compounds have been identified as a novel and precise inhibitors of EBOV infection. After *in vitro* and in vivo analysis of SERMs, Clomiphene and Toremifene were identified as effective inhibitors of EBOV infection. Both the drugs demonstrated inhibitory activity against EBOV even in the absence of evident oestrogen receptor expression. Moreover, both SERMs hindered virus entry after internalization, signifying that Clomiphene and Toremifene do not involve any of the classical pathways related to the oestrogen receptors (ERs)[43]. Another study screened 600 approved FDA drugs, out of which, 23 approved compounds were selected and divided into six categories, having a concept of blocking virus-like particle entry mediated by GP spikes into the host cells[44]. One of the most potent groups for anti-EBOV entry comprises of microtubule inhibitors, including Vinblastine, Vinorelbine, Vincristine, Colchicine, Nocodazole, Mebendazole, and Albendazole. Nocodazole's action is assumed to be associated with microtubule depolymerization, leading to

Table 2. Existing drugs of EBOV with their clinical trial phases.

Company	Commercial name	Mechanism of action	Probable target	Clinical trial status	References
Tekmira Pharmaceuticals in collaboration with the Department of Defense, Colombia	TKM-100802 TKM-130803	A small interfering RNA lipid and nanoparticle therapeutic or Cocktail of 3 small interfering RNAs (siRNAs) in lipid nanoparticle target the viral proteinsVp24 VP35 and L polymerase to silence viral genes	Zaire Ebola viral polymerase (L, VP24, VP35)	Phase Ⅰ Phase Ⅱ	[54,55]
BioCryst Pharmaceuticals Durham, USA	BCX4430	It is an adenosineanalog, which can also constrain viral replication	Viral polymerase	Phase Ⅰ	[54,56,57]
Sarepta Therapeutics, USA	AVI-6002 AVI-7537	phosphomorpholino oligonucleotide blocks viral protein production	Viral polymeras (VP24/VP35), VP24	Phase Ⅰ Phase Ⅰ	[54]
Mapp Biopharmaceutical, USA	ZMapp	It is Triple monoclonal antibody cocktail neutralizes the virus and kills infected cells	both distinct and overlapping portions of the EBOV GP	PREVAIL Ⅱ Phase Ⅰ/Ⅱ	[55,58]
Toyama Chemical, Japan	Favipiravir (T-705)	Is known as avigan and demonstrated anti-viral activities against other RNA viruses	Act Against Zaire EBOV by inhibiting the replication of the viral genome, viral polymerase	Phase Ⅱ Approved for IAV JIKI	[54,56,59]
Chimerix Inc., USA	brincidofovir (CMX001)	broad-spectrum activity against double-stranded DNA (dsDNA)	Unknown	Phase Ⅱ	[37]
Gilead Sciences, USA	Remdesivir (GS-5734)	It is a prodrug of adenosine analog and requires metabolism by the host cell to the pharmacologically active triphosphate (TP) to inhibit virus replication	Act against Zaire and Sudan species of EBOV by disrupting a key enzyme of virus essentials for replication	Phase Ⅰ	[57, 60]

the inhibition of viral entrance. The second category includes ER modulators, including Clomiphene, Raloxifene, and Toremifene. These drugs are available as an oral drug with good safety and tolerability profiles. Good plasma exposure and bioavailability make these drugs excellent contenders for the treatment of EBOV infection. The third category of compounds includes Clemastine, Maprotiline, and Benztropine, which have antihistamine and anticholinergic activities. The antipsychotic/antidepressant drugs Clomipramine, Thiothixene, and Trifluoperazine constitute the fourth category. The fifth category includes the pump/channel blockers, such as Digoxin, Dronedarone, and Propafenone. However, the potencies of these drugs are relatively weak for EBOV infection. The sixth category includes anticancer medicines and antibiotics. This category comprises of Sunitinib (multi-kinase inhibitor), Daunomycin (intercalates DNA, inhibiting DNA biosynthesis), Azithromycin and Clarithromycin (block bacterial protein synthesis). However, how these drugs can affect Ebola virus-like particle entry remains to be elucidated.

The most potent strategy for containing EBOV infection is to directly target the critical stages of the viral life cycle, which include the binding and entry into the host cell, replication, packaging, and finally release of viral progeny from host cells. Some drugs that are under different phases of a human clinical trial for the treatment of EBOV are included in Table 2. These drugs either interfere with viral replication by interfering with RNA polymerase L (*e.g.*, BCX4430) or affect the viral translational process by acting as siRNAs (*e.g.*, TKM-100802) and phosphorodiamidate morpholino oligomers (*e.g.*, AVI-6002) or by neutralizing the virus through the use of antibodies against surface expressed protein, GP (*e.g.*, ZMapp antibody cocktail). Recently, drugs that target the host proteins, utilized by EBOV to enter and replicate in the cells, have been under development [*e.g.*, cathepsins, NPC1, and T-cell immunoglobulin and mucin domain-1 (TIM-1)][45]. Cysteine proteases (such as CatB and CatL), responsible for the cleavage of EBOV GP in the endosome before fusion and entry, are being targeted for therapeutic purpose. Several proteases and cathepsin inhibitors have shown great potential against EBOV *in vitro*[46]. However, whether targeting cathepsins could be utilized for therapeutics purpose without effecting compensatory mechanisms is unknown. Inhibiting the binding of EBOV GP to NPC1 by two small molecules, MBX2254 and MBX2270, successfully prevented the infection *in vitro*[47]. Similarly, inhibiting the binding of TIM-1 to GP by the TIM-1 antibody ARD5 barred EBOV infection[48]. Another strategy that is being considered is modulation of the immune system. Modulations of cytokines, chemokines, and other proteins might either minimize the augmented inflammatory cytokine release linked with EBOV or encourage viral clearance. Post-exposure administration of adenovirus-vectored interferon (IFN)-α with ZMab antibody in nonhuman primates (NHPs) improved the survival rate and reduced the viral loads[49]. Similarly, early post-exposure administration of IFN-β caused prolonged survival of NHPs[50]. Therapies that could be helpful in managing post-EBOV infection such as reducing coagulation abnormalities and haemorrhagic manifestations are also tried. Recombinant human activated protein C and recombinant nematode anticoagulant protein c2 were administered to NHPs and their potential as therapeutic regimens were investigated[51,52]. Treatment of FX06, a fibrin-derived peptide under clinical investigation for vascular leak syndrome, to a patient having EVD associated vascular leakage and multiorgan failure substantially improved the patient condition[53].

7. Current efforts on EBOV vaccine

A few promising EBOV vaccines existed even before the West African epidemic. However, due to limited recurrence of EVD cases and intermittent nature of outbreaks of EBOV, only few pharma companies and research organizations have taken an interest in developing a potential vaccine candidate. Moreover, given the restricted market and absence of money-related motivators, vaccine development against EBOV has never been taken seriously. However, after the devastating 2014 outbreak of EVD, the scenario changed and various pharma companies, government organizations, researchers and funders took the initiative to develop EBOV vaccines. Even development of a vaccine against EBOV was of utmost importance as frontline health workers needed immunization to get them protected from the risk of infection and death. Since the discovery of EBOV, some vaccines have been developed, and few of them are now under various phases of human clinical trials (Table 3). As of 11 November 2018, forty-one completed trails, nine active and not recruiting and seven recruiting EBOV vaccine studies are listed on ClinicalTrials.gov. Out of 41 completed investigations, four studies have been updated with results. Available vaccines against EBOV utilize a variety of different platforms including recombinant viral vectors, recombinant proteins, subunit vaccines, virus-like particles and DNA vaccines (Table 3). A successful vaccine candidate should demonstrate efficacy in preclinical and clinical trials and offer immunogenicity and putative protective level against infection.

As listed in Table 3, some vaccines against EBOV are undergoing different phases of human clinical trials; however, various limitations related to the human clinical trials are a cause of concern for their efficacy in humans[61]. For instance, data associated with children is limited. During the West Africa outbreak, 21% of the patients were children (16 years or under), and a fatality rate of 80% was recorded for children age five years or younger[62]. Relying on this data, vaccination was carried out in children as young as one year of

Table 3. Possible EBOV vaccines with their human clinical trial phases.

Co-ordinator	Vaccines name	Multivalent/ Monovalent	Composition	Species of EBOV (act against)	Immune-gen	Current clinical trial status	References
GlaxoSmithKline Biologicals, BE and PHAC, CANADA	ChAd3-ZEBOV	Monovalent	Recombinant chimpanzee adenovirus serotype 3	ZEBOV	GP, NP	Phase Ⅱb/Ⅲ	[54, 66, 67]
National Institute of Allergy and Infectious Diseases, USA	ChAd3	Bivalent	Non-replicative, recombinant chimpanzee adenovirus serotype 3	SEBOV and ZEBOV (Mayinga strain)	GP	Phase 1	[68]
University of Oxford, UK and National Institute of Allergy and Infectious Diseases, USA	ChAd3-EBOZ & MVA-BN-Filo (prime/boost)	Monovalent	Non-replicative, recombinant chimpanzee adenovirus serotype 3	ZEBOV (Mayinga strain)	GP	Phase 1	[68]
NewLink Genetics and Merck Vaccines, USA	rVSV-ZEBOVor recombinant vesicular stomatitis virus	Monovalent	Recombinant vesicular stomatitis virus	ZEBOV (Mayinga strain)	GP	Phase Ⅲ	[66, 68-70]
ProfectusBioSciences, USA	rVSV N4CT1 EBOVGP1	Trivalent	recombinant vesicular stomatitis virus	ZEBOV (Mayinga strain), SEBOV and Marburg	GP	Phase Ⅰ	[68]
Gamaleya Research Institute for Epidemiology and Microbiology, Russia	GamEvac-Combi (rVSV& Ad5, prime/boost)	Monovalent	Replicative, heterologous recombinant vesicular stomatitis virus and human adenovirus serotype 5	ZEBOV (Makona strain) (prime & heterologous boost)	GP	Phase Ⅰ/Ⅱ &Ⅳ On 28/12/2016, MOH of Russian Federation registered vaccine (no. LP-003390)	[68]
CanSino Biologics & Beijing Institute of Biotechnology, China	Ad5-EBOV	Monovalent	Non-replicative, recombinant human adenovirus serotype 5	ZEBOV (Makona strain)	GP	Phase Ⅰ/Ⅱ	[68]
Janssen Vaccines & Prevention B.V, Netherlands	Ad26-EBOV/ MVAEBOV Prime/boost	Pentavalent	Non-replicative, recombinant adenovirus serotype 26 and modified vaccinia Ankara expressing fourfiloviruses nucleoproteins	ZEBOV (Mayinga strain), SEBOV, and Marburg viruses and nucleoprotein of TEBOV	GP	Phase Ⅱb/Ⅲ	[66, 71]
Novavax, USA	EBOV GP nanoparticle recombinant vaccine	Monovalent	Baculovirus-derived Ebola GP nanoparticles with a Matrix M adjuvant	ZBOV (Makona strain)	Anti-GP IgG responses	Phase Ⅰ	[66, 68, 72]
Thomas Jefferson University, USA	Rabies EBOV-GP	Trivalent	Replicates a competent live virus vaccine trivalent (Zaire, Sudan, Marburg) + Rabies-vector backbone used in HIV-1 vaccine candidates	SEBOV ZEBOV (Mayinga strain)	GP	Phase Ⅰ	[70]
Vaxart, USA	Oral Ad5 (Oral human adenovirus-based Ebola candidate (tablet vaccine)	Monovalent	A disabled virus (non-replicating adenovirus type 5, or Ad5) that co-delivers the gene for the specific vaccine antigen and the gene for a TLR3 ligand that functions as an adjuvant to amplify the immune response	SEBOV	GP	Phase Ⅰ	[66, 67, 73]
InovioPharmaceuticals, USA	DNA-EBOV with electroporation	Multivalent	INO-4212 (with 2 components INO-4201 [past Ebola Zaire virus outbreak strains] & INO-4202 [2014–2015 Ebola Zaire virus outbreak strains])	EBOV, MARV, SEBOV and Ravn virus	GP	Phase Ⅰ	[68, 73, 74]
VaxArt, USA and LONZA biological, USA	VXA ZEBOV-GP	Monovalent	Recombinant VSV-VECTOR vaccine used in HIV-1 vaccine candidates. Using replication competent live oral monovalent virus vaccine	ZEBOV, 1995 strain	GP	Phase 1	[75]

age during the 2018 EBOV epidemic in the Democratic Republic of the Congo. Another limitation is from the inadequate data available from the pregnant women. During clinical trials, pregnant women are usually excluded; in the 2018 EBOV outbreak in the Democratic Republic of the Congo, they were omitted from the vaccination programme. Another probable limitation that should be considered during the development of vaccines is immune-compromised populations such as HIV-infected and elderly population. The vaccination regime may have varied response in these populations compared to healthy ones. Apart from the discussed limitations, a number of variables such as long-term protections (clinical data is only available until 24 months after vaccination), rapidity of immune response and protection (EBOV spreads quickly through contact to contact), a correlation between immune response and clinical defence, any vaccine-related adverse events and a trust among the ongoing clinical trials and the participants should be carefully considered. To overcome these hurdles, recently, the Partnership for Research on Ebola Vaccinations was established as an international consortium. The consortium includes various research and academic institutions such as the French Institute for Health and Medical Research (Inserm), the US National Institutes of Health, London School of Hygiene & Tropical Medicine, and the Universities of Bordeaux and Minnesota. Additionally, health establishments and researchers from four EBOV-affected countries (Guinea, Liberia, Mali and Sierra Leone), nongovernmental organizations such as the Alliance for International Medical Action and Leidos and pharmaceutical companies (Johnson & Johnson, Merck, and Bavarian Nordic) are also participating. Presently, the consortium is carrying out a randomized, double-blind, placebo-controlled clinical trial of three EBOV vaccines in adults and children (less than one year). The trail vaccines include 1) recombinant vesicular stomatitis virus (rVSV)-ZEBOV prime minus any boost, 2) rVSV-ZEBOV prime with a rVSV-ZEBOV boost and 3) adenovirus serotype-26(Ad26).ZEBOV prime with an MVA-BN-Filo boost[61].

Efforts are being made for licensure of the first EBOV vaccines in various countries (the European Union, Africa, and the United States), but of course, approval may differ as per regulatory agencies. Apart from these countries, Ad5-EBOV vaccine (expressing EBOV GP) developed by Chinese Academy of Military Medical Sciences' Bioengineering Institute and National Research Council of Canada and produced by CanSinoBio has been authorized in China. Another vaccine, GamEvac-Combi, a heterologous VSV- and Ad5-vectored prime-boost EBOV vaccine (expressing EBOV GP), has been approved in Russia. However, as discussed earlier, limited information about the immunogenicity and safety of these two vaccines in humans is available[63,64]. Recently, a study utilized available structural data of the prefusion state of EBOV GP at low and high-resolution and used a computational approach of Motif-Matching Fragment Assembly method (MMFA) to describe the fusion state structures of viral entry. This allowed them to create atomic-level models of EBOV GPs in the fusion state. Based on the generated models, the study determined that the antibody KZ522 prevents viral entry by blocking the prefusion-to-fusion transition. Antibody mAb100 blocked not only the development, but also the exposure of the fusion peptide when Ebola GP was in the fusion state. The modelling approach utilized by the study provides a framework for developing structure-based knowledge. Data acquired will be helpful for not only understanding the sequence-structure relationships but also the protein-protein interactions and functions, assisting in vaccine design for viruses and other pathogens[65].

8. Future perspectives and concluding remarks

Regardless of the development in the field of drugs and vaccines against EBOV, the world is still not adequately prepared for future epidemics. After an outbreak is effectively controlled, strict control measures are required to curb any spread of infection from the affected patient. In the absence of FDA-approved drugs and licensed vaccines with tested efficacy, a cure/protection against EVD is still unavailable. Hence, strategies that could contain the spread of EVD during an outbreak need more attention and improvement at the community level. This includes fast identification and isolation of the infected, strict control measures at hospitals, continuous follow-up of the infected, and most importantly, safe burials practices. In addition, awareness programmes should be planned on a large scale to educate people about the disease for its containment and eradication. Development of simple and rapid diagnostic kits would be essential to carry out preliminary screening of the infection and thus requires more scientific effort. Current drug discovery efforts are anticipated to result in the development of formulations that will be readily available and affordable for the treatment of EVD. However, this process is time consuming and is often beleaguered by high costs and fluctuating attrition rates. Moreover, only a few of the many compounds evaluated in the initial discovery stages will ever make it to the clinic.

The drugs that are currently under different phases of clinical trials are also suffering from various limitations. For example, most of the early trials for the drugs are carried out in an area that is distinct from the sites of the actual outbreaks. Hence, owing to the genetic and immunological variances among dissimilar patient populations, care should be taken while interpreting data from Phase I trials. Also, the affected patients within the outbreak regions are susceptible to disease such as malaria, having symptoms similar to EBOV infection (fever, headache, fatigue, muscle pain, vomiting, *etc*.). Administrations of any antimalarial medication along with EBOV

drug candidate may result in drug-drug interactions and thus might confuse phase Ⅱ/Ⅲ outcomes. A vast majority of developed drugs for EBOV treatment are focused on EBOV Zaire. So, efforts should be made to develop therapeutics with broad-spectrum activity. For instance, GS-5734 is effective against both EBOV and MARV, whereas drugs such as BCX4430 with activity against several RNA viruses are promising candidates[76]. Some vaccines are undergoing various stages of human clinical and experimental trials but due to various limitations of testing efficacy and sporadic nature of outbreaks, getting them authorized by WHO or the nations where the outbreak occurs is difficult. Considering the current scenario, great efforts with clear insight would be needed for developing potential drugs and vaccines for the treatment of patients with EVD.

Conflict of interest statement

We declare that we have no conflict of interest.

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