

*Review Article***Ebola Virus Disease: Biology, Diagnosis, Treatment and Prevention of Epidemics**SUSHIL KUMAR^{1,2,*}, RENU KUMARI², RICHA PANDEY³ and VISHAKHA SHARMA^{1,2}¹SKA Institution for Research, Education and Development (SKAIRE), 4/11 Sarv Priya Vihar, New Delhi 110 016, India²National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110 067, India³Division of Natural Products Chemistry, CSIR-Indian Institute of Chemical Technology, Uppal Road, Tarnaka Uppal Road, Hyderabad 500 007, India

(Received on 24 October 2015; Revised on 16 April 2016; Re-revised on 28 May 2016; Accepted on 20 October 2016)

Ebola virus disease (EVD) is a highly lethal contagious disease caused by the negative RNA strand Ebola virus. Reservoir in wild forest animals of Africa, Ebola virus infects humans that come in direct contact of diseased animals and outbreaks of EVD result from person to person spread of infection. A recent EVD outbreak in West Africa has killed several thousand persons. Since Ebola infection will persist in animals, EVD epidemics are expected to continue to occur in future. Infected travellers from Africa can initiate/import outbreaks in countries of other continents. This review describes the properties of the Ebola virus and EVD, the ongoing attempts to develop diagnostics, vaccines and medicines for prevention and cure of EVD and the supportive care that saves some EVD patients. Also discussed are measures that can stop and prevent EVD outbreaks. Need for inclusion of EVD in the education, research, drug and medical equipment manufacturing programmes, of the densely populated countries such as India, is emphasised.

Keywords: Anti-Ebola Therapeutics/Vaccines; Cytokine Storm; Ebolavirus Disease; Ebola Genetics; Filovirus; Repurposed Drugs

Introduction

In addition to chikungunya, dengue, swine flu and zika viral diseases, Ebola virus disease (EVD) is a potential public health threat of pandemic proportions for India. It is so, on account of human to human transmission of the Ebola virus via exudates of patients, absence of licensed vaccine(s) for protection against the disease and of therapeutics for the treatment of disease, continued presence of Ebola virus in its reservoir hosts in the endemic areas of EVD, Ebola virus possessing the properties of category – A biothreat pathogen and high fatality rates in its patients. Since 1976 when EVD was first described, there have been at least 26 outbreaks of EVD in the Central and Western regions of Africa. Out of the two recent EVD outbreaks, smaller one in the Democratic Republic of Congo (WHO 2014a), the larger one in West Africa in a region comprising of Guinea, Liberia and Sierra

Leone (WHO 2014b) is still in progression and by July 19, 2015, about 11,269 EVD patients had died. Travelers and evacuees from the outbreak region in Africa have carried the disease to Mali, Senegal and Nigeria in Africa and to North America and Europe (en.m.wikipedia.org/wiki/Ebola_virus_e11-11-2014). One infected person or animal can spread the EVD infection in crowded location(s) such that outbreaks thereafter can assume pandemicity. Unless the invasion of EVD is controlled by all-round preparedness, EVD in India, if it some how gets introduced, could rapidly become a pandemic. Preventive measures against the emerging Zika virus disease (ZVD) are being developed using those enunciated against the EVD as the model (Currie *et al.*, 2016).

In view of the EVD outbreaks and Ebola virus emerging as a potent bioweapon in recent years the

*Author for Correspondence: E-mail: sushil2000_01@yahoo.co.in

research on various aspects of the EVD has been growing steadily, internationally. In the present article, some of the important information about the Ebola virus and EVD described in the current scientific literature has been summarized and discussed to serve as introduction on the subject, with the hope that it will spur greater interest and some research activity on EVD in India, latter especially in the areas of EVD rapid diagnosis, therapeutics and vaccines and other logistics of EVD control.

Ebola Virus Characteristics

Taxonomy, Morphology and Structure

Ebola belongs to the order mononegavirales that comprises of viruses, in which the genome consists of non-segmented (single) negative strand RNA (Kuhn *et al.*, 2010; Li and Chen 2014; International Committee on Taxonomy 2013). Four families, having a common ancestor, make the order mononegavirales: Filoviridae (exemplified by the ebola virus), Rhabdoviridae (includes the rabies virus), Paramyxoviridae (viruses that cause mumps and measles) and Bornaviridae (that cause disease in horses and other mammalian animals). Besides ebola, the other filoviral genera are Marburg virus and Cueva virus. Marburg virus causes a haemorrhagic disease in humans different from EVD. Ebola and Marburg viruses are highly virulent (perhaps most virulent) pathogens such that they can cause death in humans in 6 to 16 days from infection (Mahanty and Bray 2004; Macleod 2010; Leroy *et al.*, 2011a, b; Funk and Kumar 2015). Unlike Marburg virus, Ebola is not called a hemorrhagic fever virus since EVD patients develop bleeding rarely, usually in the terminal phase and not earlier (Fowler *et al.*, 2014). Filoviruses are believed to have originated from an ancestor about 16-23 million years ago, coinciding with the origin of great apes (Taylor *et al.*, 2014). There is evidence that new strains (species) of virus emerge during passage in animals and humans (Kuhn *et al.*, 2014). Ebola virions are tubular, generally 80 nM in diameter and 800 nM in length; however, due to concatamerization some ebola virions may be as long as 14000 nM. They are pleomorphic by being linear, branched, V-shaped or 6-shaped (Sanchez 2001; Acha and Szyfres 2003). The single, linear, helical and negative sense RNA genome is contained in a nucleocapsid, which is enveloped by another capsid,

which in turn is enclosed in a lipid membrane. There are knob-shaped surface projections on the virus, 10nM long and placed 10nM apart as peplomes embedded in the outer surface of the lipid bilayer (Fig. 1A and 2A). The genus ebola has species, that have different geographical origins and demonstrate 3 to 41% nucleic acid sequence divergence; the species are named after the geographical region of their original identification (Fauci 2014; Kuhn *et al.*, 2014). The ebola species can be arranged in the following order based on their pathogenicity-cum-virulence: Zaire ebola virus (ZEBOV) > Guinea ebola virus (GEBOV, responsible for the ongoing EVD epidemic in West Africa) > Sudan ebola virus (TAFV) > Reston ebola virus (REBOV, non-pathogenic on humans) (Cheng and Kelly 2014; Spickler 2014). ZEBOV is the type species and is also referred simply as EBOV. Genetics of differential pathogenicity between EBOV species needs dissection.

Host Range

EVD is a highly contagious zoonotic disease, since all its outbreaks are believed to have started by the transmission of Ebola virus to humans via vertebrate animals; human to human transmissions of Ebola are secondary infections. Humans get infected when they come in contact with blood or body fluids or eat meat of infected animals. Rise in population and deforestation have increased the contact of humans with infected wild life (Bausch and Schwarz 2014; Walsh and Haseeb 2015). People living in the Ebola-affected as well as unaffected villages in Africa carry antibodies against Ebola in the range of 1.8 to 21.3 %

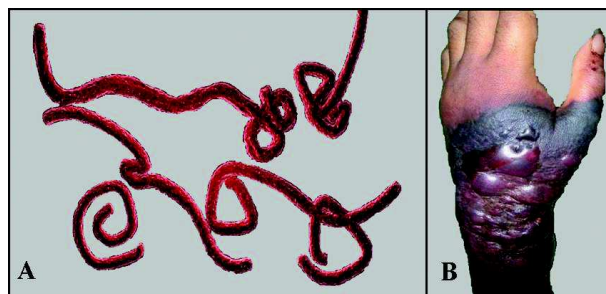


Fig. 1: Ebola virus and the hemorrhagic disease caused by it. A = Ebola virus particles (http://www.biomagazine.gr/site_data/articles/2014053120104754.jpg); B = Hand of an infected person showing a type of lesion/symptom produced by the Ebola virus disease (<http://absolute-news.com/wp-Content/uploads/2014/ebola-hand1.jpg>)

(Leroy *et al.*, 2000). There is evidence that the present epidemic of EVD in progress in West Africa started in December 2013 as a zoonotic transmission to a two years old boy, in the village Gueckedou in Guinea from Ebola harboring *Hypsignathus monstrosus* and *Epomops franqueti* bats, infection spread therefrom person to person nosocomially (Table 1; en.m.wikipedia.org/wiki/Ebola_virus_e;10-11-2014; Baize *et al.*, 2014). Since bats upon screening were often found to possess Ebola specific antibodies and RNA, bats have been presumed to be the natural reservoir of the virus in Africa; bats get infected with Ebola, lack overt disease and survive (Swanepoel *et al.*, 1996; Reiter *et al.*, 1999; Leroy *et al.*, 2004 and 2005; Pourrut *et al.*, 2005 and 2007; Peterson *et al.*, 2007; Olival *et al.*, 2013; Baize *et al.*, 2014; Ng *et al.*, 2015). Presence of Ebola antibodies has also been noted among bat populations in China, Bangladesh and Phillipines (Olival and Hayman 2014). Ebola antibodies and/or RNA have also been found in the bodies of a variety of non-bat vertebrates, including non-human primates, monkeys, cats, foxes, hogs, antelopes, porcupines and rodents (www.publichealth.gc.ca). It is thought that in forests infested with Ebola infected bats, a variety of co-inhabiting vertebrates catch Ebola infection by coming in contact or eating food (dead or live animals and fruits, flowers and leaves) on which bats have drooled or defecated and which has been similarly contaminated by the body fluids of infected non-bat animals or by eating of meat of the dead or alive Ebola diseased animals. There could be reservoir species of Ebola other than bats. Table 1 gives a list of wild, domesticated and laboratory model animals that get infected with Ebola, but may or may not die of the Ebola disease caused in them. Ebola virus titer is very high in lungs of some infected animals such as pigs which can transmit Ebola virus to other animals via air; however, airborne transmission may not occur in humans since virus titers in lungs are lower than in blood (Weingartl *et al.*, 2013). In humans there are possibilities of transmission through air over short distance (Wong *et al.*, 2015). Ebola virus is principally an animalspecific virus which has assumed high level virulence by shifting to new hosts such as humans (Longdon *et al.*, 2015).

Transmission Among Humans

Ebola enters humans through nose, mouth, eyes, ears, open wounds, cuts and abraded skin or mucous

membranes, via direct or indirect contact with the body fluids of a person who has developed the symptoms of EVD or has died from it. The blood, vomit, urine, faeces, sweat, tears, breast milk, semen, mucus, saliva, spit and any other kind of fluid from humans having EVD contains infective Ebola virus. Ebola is present on the skin of EVD patients or their cadavers and there touching them can result in infection. Ebola infection can also result from exposure to saliva from a coughing EVD patients. Contaminated needles and medical equipment if reused without sterilization can cause Ebola infection. Ebola can survive for upto several weeks on eating utensils, bedding, clothing, furniture, door knobs, electrical switches and such materials that may get contaminated by the body fluids and also in water into which body fluids may have been washed into, from the EVD patients (Bibby *et al.*, 2015). Ebola virus remains in the body of survivors for many months after disease has subsided, especially in immunologically protected organs such as eyes and testicles. Ebola's presence has been recorded in semen of convalescent men at 179 day (average 26 weeks) after recovery (http://www.phac_aspc.gc.ca/lab-bio/res/pds-ftss/ebpola-eng.php gives references; Rowe *et al.*, 1999; Cardona-Maya *et al.*, 2014; Mate *et al.*, 2015). Aqueous humor of uveitis eye was found to contain active Ebola viruses, 10 weeks after virus had disappeared from a patient's blood (Varkey *et al.*, 2015). Transmission from touching the diseased body is possible for at least seven days post-mortem (Prescott *et al.*, 2015). Virus RNA remains in cadavers for months but naked -ve sense RNA is not infective. Genome sequence analysis of 318 patients in Sierra Leone conclusively demonstrated the a single introduction of Ebola into human population was responsible for the epidemic and that it resulted from human to human transmission (Park *et al.*, 2015).

Genetics

Genome, Gene Functions, Transcription and Replication

The Ebolavirus genome is a 19 kb single stranded RNA molecule of negative polarity. The seven genes encoded in it lie in the order 3'-Leader-NP-VP35-VP40-GP/sGP-VP30-VP24-L-Trailer-5', as shown in Figure 2A and B. The gene functions are summarized in the Table 2. GP1,2, VP24, VP40, VP30,

VP35, NP and L are not only virion structural proteins but also possess a variety of enzymatic and/or regulatory properties for the processes of virion attachment on host cells, virion entry into host cell cytoplasm and viral multiplication (Fig. 2C), arrest of antiviral host cell responses, host cell apoptosis and other pathophysiological changes in the host to build a vast reservoir of virus particles for the spread of infection among susceptible animals and humans (Fig. 3).

The *cis* sequences contained in Leader and Trailer regions are essential signals for the control of gene transcription, genome replication and packaging of replicated RNA into virus particles (Crary et al., 2003). Each of the seven genes has its own transcription initiation and termination signals flanking it. The open reading frame of each gene, containing its coding region, is flanked by non-translated sequences of unknown function (Klenk and Feldmann

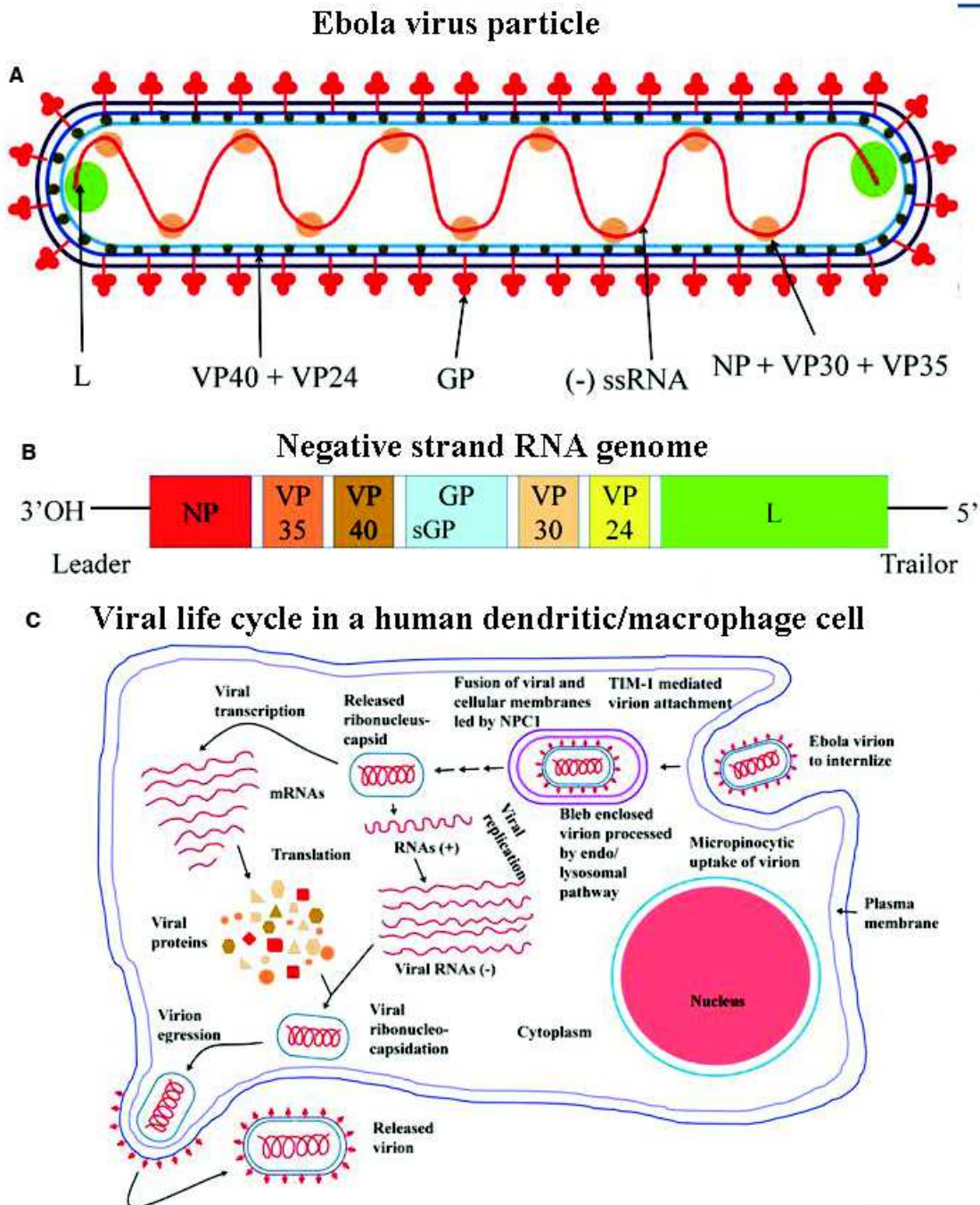


Fig. 2: Caption on next page

Fig. 2: Diagrammatic representation of Ebola virus particle (A), its negative single stranded RNA genome (B) and its growth cycle in a human dendritic cell (C). A = The virion is tubular, about 80 nm in diameter and 970 nm in length. Outermost envelope is composed of host cell membrane bearing 10 nm long viral glycoprotein (GP) studs; the studs trimeric in structure consist of disulphide linked GP1 and GP2 the furin cleaved fragments of GP. Inside the envelope lies the nucleocapsid, which consists of a single stranded helical RNA molecule about which are wrapped the viral proteins nucleoprotein (NP), minor nucleoprotein VP30 and polymerase cofactor VP35. The RNA dependent RNA polymerase (RDRP) or L protein is also present on the RNA genome. The RNA capsule is bounded by a membrane comprising of the matrix protein VP40 and membrane associated protein VP24. B = The genome is unsegmented, a polycistronic RNA that specifies seven proteins in the 3' to 5' order of NP, VP35, VP40, GP/sGP, VP30, VP24 and L (RDRP). The sGP is truncated soluble protein GP. The VP30 transcription anti-terminator facilitates continued transcription of genes from the gene NP to gene L. Each gene has its own transcription initiation and termination sequences and translation initiation and stop codons. During transcription initiation RDRP, the product of L gene binds to the Leader sequence. During replication RDRP binds to the Trailer sequence of antigenome (+) copy of RNA. C = The virus attaches to the host cell by binding of its GP protein to the host cell receptors in plasma membrane such as T-cell immunoglobulin, mucin domain protein (TIM-1) and mannose binding trans-membrane C-type lectins. Virus particle is internalized by the endo/lysosomal pathway led macropinocytosis and gets endocytosed into a bleb/ruffle/vesicle. The Niemann Pick C1 (NPC1) protein of human cell membrane binds to the GP protein of the virus. This, together with endosomal acid pH and other multiple factors trigger the fusion of viral membrane with cytoplasmic membrane of vesicle resulting in the release of viral nucleocapsid into host cell cytoplasm. RDRP binds to the leader sequence of the ssRNA genome of virus and initiates transcription. mRNAs of one to seven gene sizes are synthesized and translated such that NP and L are respectively the most and least abundant proteins formed. This happens because in between genes are present the transcription stop signal followed by the transcription anti-terminator signal. For replication RDRP binds to the Leader sequence and produces antigenome (+)ve ssRNAs. The encapsidated (+)ve RNA strands serve as templates for RDRP to produce (-)ve RNA strands. In the replication process RDRP binds to the Trailer sequence and copies the (+)ve strand templates into (-)ve strand RNAs. The (-)ve ssRNA progeny strands get encapsidated. The completely assembled virion in host cytoplasm has ssRNA, NP, VP30, VP35, VP24, VP40 and L. Finally, the encapsidated virions arrive at the plasma membrane, where budding occurs. The budding process allows the particles to obtain their outer envelope from the cellular membrane, which then gets studded by the GP protein. The mature virus particles so produced can initiate a new cycle of infection. (Klenk and Feldmann 2004; Hartlieb and Weissenhorn 2006; Muhlberger 2007; Ascenzi *et al.*, 2008; Hartman *et al.*, 2008; Hoenen *et al.*, 2010; Liu *et al.*, 2010; Cote *et al.*, 2011; Feldmann and Geisbert 2011; Leroy *et al.*, 2011; Mehedi *et al.*, 2011; Olejnik *et al.*, 2011; Miller *et al.*, 2012; Miller and Chandran 2012; Moller-Tank *et al.*, 2013; www.micro.msb.le.uk/3035/Filoviruses.html, www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm and www.genome.ucsc.edu/Ebolaportal; Chiappelli *et al.*, 2015; de La Vega *et al.*, 2015; Jun *et al.*, 2015; Rhein and Maury 2015)

2004; Jasenosky and Kawaoka 2004; Hartlieb and Weissenhorn 2006). Ebola genome transcription occurs in the host cell cytoplasm after the nucleocapsid of the virion has partially uncoated. In the nucleocapsid, RNA genome is associated with the products of NP, L, VP35 and VP30 genes forming a ribonucleoprotein (RNP) complex. In the RNP, expression begins when the L gene product RNA dependent RNA polymerase (RDRP) transcribes the Leader into a 5'-triphosphate Leader RNA and stops. RDRP restarts at the transcription start signal of NP gene. The initiated NP mRNA is capped. At the NP gene transcription termination site, before NP mRNA is released, the RDRP stutters at a stretch of Us and produces a polyadenylated tail on NP mRNA. Then RDRP moves on to transcribe the gene VP35. Seven genes are transcribed sequentially in the order of their arrangement on Ebola genome.

Interestingly, the GP gene transcription results in production of mRNA for three different gene

products, namely Pre-sGP (small GP), Pre-GP and Pre-ssGP (small secretory GP), which post-translation get respectively processed into sGP, GP1 and GP2, and ssGP. Normal transcription (bulk) produces mRNA for Pre-sGP, which is the GP gene's primary product ($\geq 70\%$). Editing in transcription (editing = RDRP reads a template base more than once causing a base addition in the mRNA product) produces Pre-GP ($\leq 25\%$) and ssGP (minor, $\sim 5\%$) mRNAs. At the editing prone site, insertion of an additional A residue at the RNA editing site results in mRNA for Pre-GP protein. Likewise, insertion of two A residues produces ssGP mRNA (Volchkov *et al.*, 1995; Sanchez *et al.*, 1996; Mehedi *et al.*, 2011; Mohan *et al.*, 2012; Mehedi *et al.*, 2013; Shabman *et al.*, 2014; Mohan *et al.*, 2015).

Genes located nearest to the Leader sequence are most transcribed and those near the Trailer sequence are less transcribed. NP is most expressed and the concentration of its protein product determines

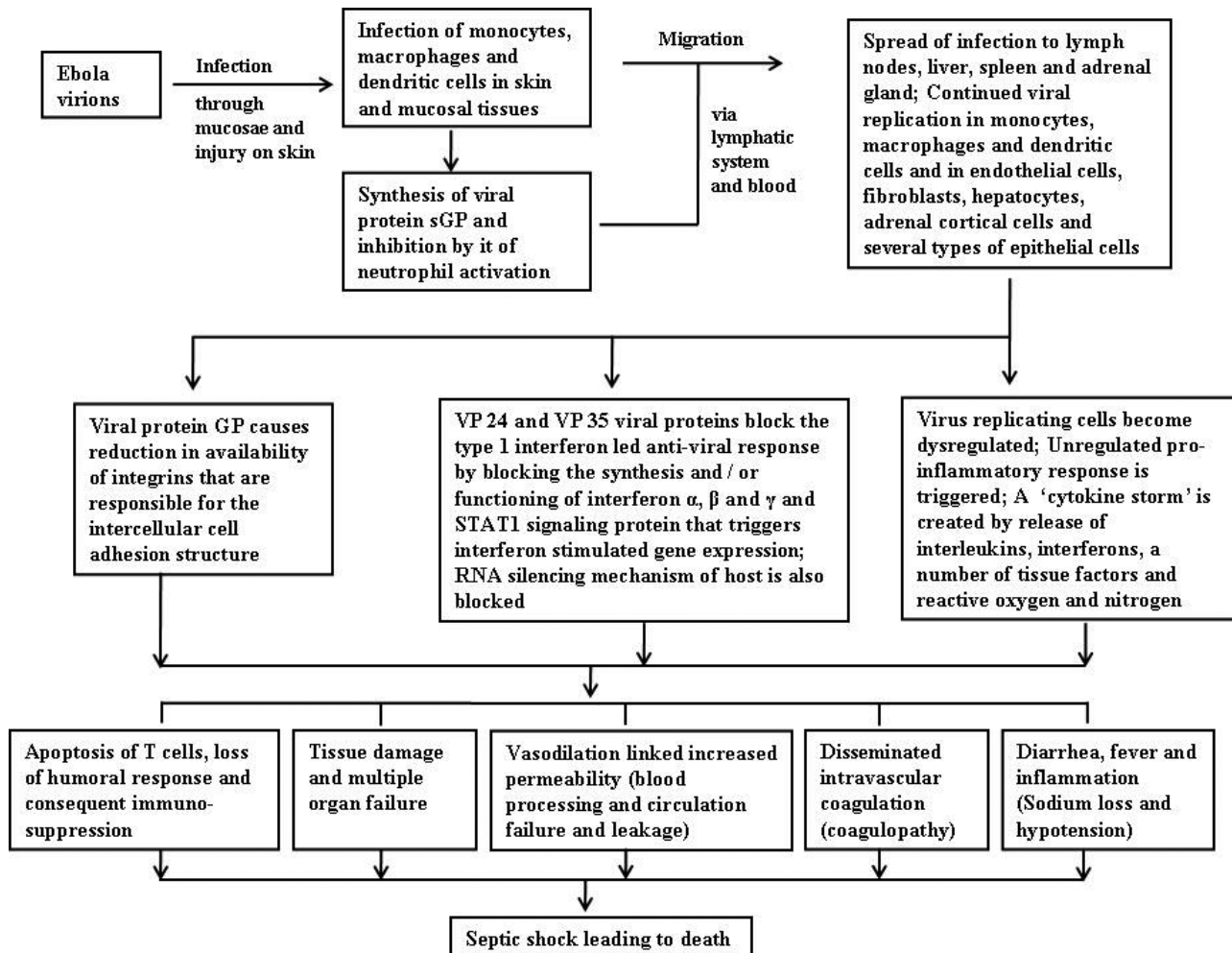


Fig. 3: Diagramme of a model of pathophysiology of Ebola virus disease in humans, based on observations on naturally infected humans and experimentally infected guineapigs, mice and macaque monkeys. Ebola viruses infect a large variety of cells in different tissues/ organs and destroy them by inhibiting the essential cellular processes. Viral proteins interact with host proteins and thereby suppress host's antiviral response. The process includes suppression of host's innate and adaptive immune response from virus led apoptosis of natural killer and T cells. There is setting in of coagulopathy and leakage of blood (hemorrhage) on account of vasodilation and increased vascular permeability as the disease progresses. Spread of infection to parenchyma cells leads to multiorgan failure. Sepsis occurs and multiple causes lead to death of Ebola infected person (References = Fisher-Hoch *et al.*, 1985; Ryabchikova *et al.*, 1999; Vilinger *et al.*, 1999; Zaki *et al.*, 1999; Basler *et al.*, 2000; Baize *et al.*, 2000; Basler *et al.*, 2003; Sullivan *et al.*, 2003; Geisbert *et al.*, 2003; Basler *et al.*, 2004; Mahanty and Bray 2004; Reed *et al.*, 2004; Smith 2005; Reid *et al.*, 2006; Sanchez *et al.*, 2006; Yaddanapudi *et al.*, 2006; Mohamadzadeh *et al.*, 2007; Reid *et al.*, 2007; Hartman *et al.*, 2008; Chang *et al.*, 2009; Kimberlin *et al.*, 2008; Bradfute *et al.*, 2010; Jin *et al.*, 2010; Wauquier *et al.*, 2010; Fabbazzi *et al.*, 2011; Olejnik *et al.*, 2011; Kuhl *et al.*, 2012; Mohan *et al.*, 2012; Luthra *et al.*, 2013; Adu-Gyamfi *et al.*, 2014; Xu *et al.*, 2014; Chiappelli *et al.*, 2015)

the switching from gene transcription to genome replication. RDRP replicates the genome by binding to the 3' Leader and moving on to the 5' end Trailer. Replication results in positive strand full length antigenomes. RDRP binds to the 5' Trailer and accomplishes full length transcription/replication of encapsidated antigenomes to produce the negative

strand genome copies. The negative single strand RNA genome copies are then encapsidated and packaged into virion progeny particles and released via host cell plasma membrane (Ascenzi *et al.*, 2008; Olejnik *et al.*, 2011; Choi and Croyle 2013).

EBOV is also dependant on several kinds of

Table 1: Host range of Ebola virus (proven and inferred, but not a complete list)

Serial order	Animal class ^a common name	Species
A	Wild animals in Central-cum-WesternAfrica forests	
1	Gorilla	<i>Gorilla gorilla</i>
2	Chimpanzee	<i>Pan troglodytes</i>
3	Monkeys	<i>Cercopithecus aethiops</i> , <i>C. hamlyni</i> , <i>C. lomamiensis</i> , <i>C. mitis</i> ; <i>Erythrocebus patas</i> ; <i>Macaca fascicularis</i> ; <i>Mandrillus sphinx</i>
4	Cat	<i>Profelis aurata</i>
5	Antelopes	<i>Cephalophus</i> sp.; <i>Tragelaphus eurycerus</i> ; <i>Orya beissa</i> , <i>O. strepsiceros</i>
6	Fox	<i>Otocyon megalotis</i>
7	Hog	<i>Hylochoerus meinertzhageni</i>
8	Racoon	<i>Procyon lotor</i>
9	Porcupine	<i>Atherurus africanus</i> ; <i>Hysterix cristata</i>
10	Dormice	<i>Graphiurus lorrainus</i>
11	Rodents/squirrels	<i>Anomalurus derbianum</i> ; <i>Dendromus mystacalis</i> ; <i>Funisciurus pyrrhopus</i> ; <i>Grammomys avidulus</i> , <i>G. dolichurus</i> , <i>G. rutilans</i> ; <i>Lemniscomys flavopunctatus</i> , <i>L. sikapusi</i> ; <i>Mus setulosus</i> ; <i>Praomys rostratus</i> , <i>P. tubbergi</i> ; <i>Protoxerus stangeri</i>
12	Shrew	<i>Sylvisores olluta</i>
13	Bats	<i>Chaerephon pumilis</i> ; <i>Epomops franqueti</i> ; <i>Eptesicus somalicus</i> ; <i>Hipposideros gigas</i> ; <i>Hypsignathus monstrosus</i> ; <i>Micropteropus pusillus</i> ; <i>Mops condylurus</i> , <i>M. nanulus</i> , <i>M. thersites</i> ; <i>Myonycteris torquata</i> ; <i>Myotis whiteleyi</i> ; <i>Myotis bocagei</i> ; <i>Pipistrellus nanus</i> ; <i>Rousettus aegyptiacus</i> ; <i>R. amplexicaudatus</i> ; <i>Scotophilus dinganoi</i> , <i>S. hirundo</i>
B	Domestic pest(s)/domesticated animals	
14	Rat	<i>Rattus rattus</i>
15	Dog	<i>Canis lupus familiaris</i>
16	Pig	<i>Sus scrofa domesticus</i>
17	Goat	<i>Capra aegagrus</i>
18	Sheep	<i>Ovis aries</i>
19	Horse	<i>Equus ferus caballus</i>
20	Buffalo	<i>Babulus bubalis</i>
21	Cow	<i>Bos primigenius</i>
C	Experimental/laboratory animals	
22	Mouse	<i>Mus musculus</i>
23	Hamster	<i>Mesocricetus auratus</i>
24	Guinea pig	<i>Cavia porcellus</i>
25	Macacque monkey	<i>Macaca mullata</i>
References	Morvan <i>et al.</i> (1999); Peterson <i>et al.</i> (2004); Allela <i>et al.</i> (2005); Leroy <i>et al.</i> (2005); Pourrut <i>et al.</i> (2005); Pourrut <i>et al.</i> (2007); Peterson <i>et al.</i> (2007); Groseth <i>et al.</i> (2007); Barrette <i>et al.</i> (2009); Leroy <i>et al.</i> (2011b); Olinger <i>et al.</i> (2012); Olival <i>et al.</i> (2013); Olival (2014) and WHO Ebola virus disease, Fact sheet no 103, September 2014; Public Health Agency of Canada (2014); Ebola Virus-Pathogen safety data sheets-www.publichealth.gc.ca	

a = Swanepoel *et al.*, (1996) and Reiter *et al.*, (1999) have demonstrated absence of Ebola virus growth in the experimentally inoculated plants of fabaceae, solanaceae, cucurbitaceae and gramineae families and in non-mammalian animals, including a few insects, spider, snail, a few reptiles and a bird

host factors for its replication. For example, the host protein eIF5A (eukaryotic initiation factor 5A), hypusinated by spermidine (a polyamine) is essential

for EBOV replication, via control of the availability of VP30 (a polymerase component) in sufficient quantity (Olsen *et al.*, 2016). The BCL2 Associated

Table 2: Gene products of the Ebola virus

S.No.	Gene name	Protein	Reference	
		Name	Function	
1	L	RNA directed RNA polymerase L	Gene/genome transcription and genome replication and formation of nucleocapsid structure	Volchkova <i>et al.</i> (1999) and Huang <i>et al.</i> (2002)
2	GP	Envelope glycoprotein: (a) small soluble glycoprotein (sGP), synthesized from a segment of GP gene, is essential; (b) GP undergoes proteolytic cleavage to produce GP1 and GP2 which bind to each other, undergo glycosylation and acylation and their trimers get inserted in viral membranous envelope	Protects GP by neutralizing the host anti-GP antibodies; acts as an anti-inflammatory factor; its delta peptide has viroporin property Essential for the attachment of virus to host cell membrane and internalization of nucleocapsid of virus into host cell cytoplasm; gives filamentous morphology to virion in cooperation with VP40; proves toxic and down regulates host cell surface proteins	Sanchez <i>et al.</i> (1996); Volchokov <i>et al.</i> (1995, 1998); Feldman <i>et al.</i> (2001); Dolnik <i>et al.</i> (2004); Chandran <i>et al.</i> (2005); Manicassamy <i>et al.</i> (2005); Wahl-Jensen <i>et al.</i> (2005); Sullivan <i>et al.</i> (2005); Marzi <i>et al.</i> (2006a, b); Falzarano <i>et al.</i> (2006); Yaddanapudi <i>et al.</i> (2006); Han <i>et al.</i> (2007); Lee <i>et al.</i> (2008); Gallaher and Garry (2015)
3	NP	Nucleoprotein NP	Essential for RNA encapsulation; NP is chaperoned by VP35 to coil and form a shell around RNA and thereby viral genome is protected by NP against host's immune response	Muhlberger <i>et al.</i> (1999); Licata <i>et al.</i> (2004); Watanabe <i>et al.</i> (2006); Dziubanska <i>et al.</i> (2014); Dong <i>et al.</i> (2015); Kirchdoerfer <i>et al.</i> (2015)
4	VP24	Membrane associated protein VP24	Anti-viral inhibitor which impairs type 1 interferon (IFN)- α/β and $-\gamma$ signalling; has a role in virus assembly and budding and in transcription and replication by being a part of nucleocapsid structure; a virulence factor that plays role in host adaptation	Huang <i>et al.</i> (2002); Han <i>et al.</i> (2003); Basler <i>et al.</i> (2004); Reid <i>et al.</i> (2006) and (2007); Hartman <i>et al.</i> (2008); Hoenen <i>et al.</i> (2010); Mateo <i>et al.</i> (2011); Kuhl and Pohlman (2012); Ebihara <i>et al.</i> (2013) and Garcia-Doriwal <i>et al.</i> (2014)
5	VP30	Minor nucleoprotein (polymerase matrix protein) VP30	Transcription antiterminator; suppression of viral RNA silencing	Haasnoot <i>et al.</i> (2007); Hartlieb <i>et al.</i> (2007); Martinez <i>et al.</i> (2008); Biedenkopf <i>et al.</i> (2013)
6	VP35	Polymerase cofactor (polymerase matrix protein) VP35	Inhibits IFN regulatory factors 3 and 7 and thereby blocks IFN- α/β gene expression; prevents anti-viral response; impedes negative control of dsRNA dependent kinase on viral replication; suppresses viral RNA silencing; is a part of virion core; binds to NP to uncoat RNA genome from virion to facilitate transcriptional expression and replication and directs newly synthesized NP to progeny RNAs	Huang <i>et al.</i> (2002); Basler <i>et al.</i> (2003); Cardenas <i>et al.</i> (2006); Feng <i>et al.</i> (2007); Haasnoot <i>et al.</i> (2007); Hartman <i>et al.</i> (2008); Chang <i>et al.</i> (2009); Prins <i>et al.</i> (2009); Schumann <i>et al.</i> (2009); Liu <i>et al.</i> (2010); Leung <i>et al.</i> (2010); Fabozzi <i>et al.</i> (2011); Ramanan <i>et al.</i> (2011); Adu-Gyamfi <i>et al.</i> (2014); Kirchdoerfer <i>et al.</i> (2015)
7	VP40	Matrix protein VP40	Required for budding of virus out of host cell membrane, links nucleocapsid and surrounding membrane and gives filamentous shape to virus together with GP and helps to maintain structural integrity of the virion	Jasenosky <i>et al.</i> (2004); Hartlieb and Weissenhorn (2006); Noda <i>et al.</i> (2002, 2007); Hoenen <i>et al.</i> (2005); Johnson <i>et al.</i> (2006); Soni <i>et al.</i> (2013)

Athanogene 3 (BAG3) product, an autophagy regulator-chaperone protein, interacts with VP40 and requesters it away from plasma membrane such that

the egress of viral particles is counteracted (Liang *et al.*, 2017). Both host-virus-interactions are targets for therapy development.

Table 3: Symptoms and laboratory variables for prognosis of the Ebola virus disease in humans

Infection stage	Clinical symptoms ^a	Laboratory variables
Very early	Fever, fatigue (prostration), headache, myalgia (muscle pain), anorexia (loss of appetite)	Leucopenia (decrease in number of total whole blood cells) with lymphopenia (less number of a type of whole blood cell) and thereafter neutrophilia (excess of neutrophils, the white blood cell type that fight infection)
Early	Nausea/vomiting, diarrhoea, cough, sore throat, nasal discharge, shortness of breath, glossitis (inflammation of tongue), gingivitis (swelling in gums) and abortion in pregnant woman	Course of infection is predictable also by the determination of killer cells in blood possessing the marker CX3CR and Ebola encoded miRNAs
Median	Minor rash (change in colour or texture of skin) on face, torso and extremities, petechiae (red spots on skin caused by intradermal hemorrhage), hearing loss, tinnitus (ringing in ears), photophobia, increased lachrymation (secretion of tears), uveitis (inflammation of uvea of eye), decreased visual acuity, edema and pain in scrotum, bleeding from mouth, nipple and ears, jaundice, mask like face, ecchymoses (hematoma caused by rupturing of blood vessel(s)), conjunctival hemorrhage (burst blood vessel in eye), bleeding from punctured sites, difficulty in breathing and swallowing, hiccups, melaena (passage of black stool), haematemeis (vomiting of blood), delirium	Thrombocytopenia (decrease in platelet count), high levels of aminotransferase in serum, hyper protein-aemia (decrease in platelet count) and proteinuria (presence of excess serum proteins in urine). Extension of prothrombin and partial thromboplastin times (measures of bleeding and blood coagulation) and detection of fibrin split products formed by blood clot degeneration. Elevation of lactate levels.
Late	Hypotension, mucosal and visceral hemorrhage, coagulopathy (impairment of blood clotting), dehydration, multiorgan failure, shock (inadequate blood flow), myocarditis, tachypnea (rapid breathing), anuria (nonpassage of urine), convulsions, coma (unconsciousness lasting for more than six hours); mortality range 50-90% ^b	
Reference(s)	Sanchez <i>et al.</i> (2006); Feldmann <i>et al.</i> (2007); Feldmann and Geisbert (2011); Fowler <i>et al.</i> (2014); Liang <i>et al.</i> (2014); Botcher <i>et al.</i> (2015); Gao <i>et al.</i> (2016); Richardson <i>et al.</i> (2016); and http://www.symptoms.com/en/info/ebola-virus-disease .	

a = All infected human do not show all the symptoms; symptoms occur in a variety of combinations; b = Some patients recover after one or two weeks; during convalescence patients may have joint pain, inflamed eyes, spinal cord and testes, hearing disability, hepatitis, psychosis (delusions), sloughing of skin and secondary infections

Evolution

The genus Ebola is surmised to have diverged from the lineages of other single negative strand viruses some 20 million years ago (MYA). There is evidence showing that specific filovirus genes got integrated in the genomes of clades of old world (Afro-Eurasia) and new world (Americas) bats, rodents and insectivore mammals, as early as 25-18 MYA (Ng *et al.*, 2015). In several species of bats, rodents and marsupials, *VP35*-, *NP*- and *L*- like Ebola genes have been detected. Only in the related species, fossil copies of Ebola genes are often present in syntenous positions; this indicates that interaction between mammals and Ebola has occurred repeatedly (Taylor *et al.*, 2011 and 2014). Ebola genes present in Ebola sensitive species may be in their wild type or mutant state. The Ebola tolerant phenotype of Ebola reservoir

bat species (Leroy *et al.*, 2005; Ng *et al.*, 2015) may be because of the animal genome borne Ebola gene products negatively complement proteins specified by the infecting Ebola virus.

Ebola genome is prone to high mutation rate, because its L protein (RDRP) makes errors during genome replication but is not able to correct them. Ebola lacks the proof reading mechanism of DNA polymerases (Liu *et al.*, 2003). The mutation rate in the Ebola *GP* gene is estimated as 3.6×10^{-5} non-synonymous substitutions/site/year (Suzuki and Gojobori 1997). For the whole genome, mutation rate in different Ebola species is known to vary from 0.45×10^{-4} to 1.25×10^{-3} nucleotide substitutions/site/year (Park *et al.*, 2015). The average molecular rate of evolution in the Ebola genus is therefore several orders of magnitude greater (3.5×10^4 times) than that in the

Table 4: Reliable, fast and simple diagnostic tests of Ebola virus infection/disease now to become available in the form of kits

Attribute	Quantitative real time reverse transcription polymerase chain reaction (qRT-PCR) ^{a,b}	Antigen-capture enzyme-linked immunosorbent assay (ELISA)	Dipstick immuno-assay called ReEBOV Antigen Rapid Test ^c	Magnetic nanoparticle-based immunochromatographic strip (Nanozyme-strip) ^e	Multiplexed lateral flow diagnostic ^c
Principle	Amplification of a specific genome segment of Ebola virus by the RNA present in the sample	Detection of Ebola specific antigen in the sample	As in ELISA	Anti-Ebola virus (EBOV) antibody coated probe recognizes, separates and visualizes EBOV on a strip	Simultaneous detection of Ebola, Yellow fever and Dengue viruses
Sample from the presumably infected human	Blood or plasma	Serum, plasma or whole blood	Plasma or whole blood, latter by finger prick	As in ELISA	Blood serum
Sterilization of the sample	Treatment with a chaotrope such as trizol or guanidium isothiocyanate	Exposure to high temperature or gamma radiation	D		
Ebola gene/protein whose presence is tested	Nucleoprotein (NP) gene domain conserved in Zaire and Sudan species of Ebola virus	NP epitope defined by a sequence of 26 aminoacids near the C terminus of NP protein	VP40 matrix protein	Glycoprotein (GP)	NS1 protein of Yellow fever and Dengue viruses and GP of Ebola virus
Whether readymade kit(s) is/are available	Yes; Newsana diagnostic test for Ebola produced by Primer Design Ltd (a 90 minutes assay); test developed by the Department of Defense, US Government; AgPath-ID One Step RT-PCR of Applied Biosystems; and EBOV Accu Power Real Time PCR kit of Bioneer	Yes; VEGA Ebola Test Device produced by Vega Medicine Ltd; Anti-Zaire-Ebola Virus Nucleoprotein (NP) IgM ELISA kit of Alpha Diagnostic International; eZYSCREEN produced by Vedalabs and Atomic Energy Commission (France) (a 15 minutes assay)	Yes; Corgenix Inc. Broomfield Co. Colorado, USA (approved by World Health Organization) (a 15 minutes test costs about \$ 15 or Rs. 1000)	A patent has been applied for and the test is being used in the field in West Africa under the auspices of Center for Disease Control (CDC)	This 15 minute test requires U.S. Food and Drug Administration approval for its commercial production
References	Leroy <i>et al.</i> (2000); Towner <i>et al.</i> (2004); Stephens <i>et al.</i> (2010); Pang <i>et al.</i> (2014); Ksiazek <i>et al.</i> (1999); Nikura <i>et al.</i> (2001); Lucht <i>et al.</i> (2003); Grolla <i>et al.</i> (2005); Broadhurst <i>et al.</i> (2015); Duan <i>et al.</i> (2015); Yen <i>et al.</i> (2015)				

a = From October to December 2014, U.S. Food and Drug Administration (www.fda.gov/MedicalDevices/Safety/E.) issued authorizations for the emergency use of the following tests: CDC Ebola Virus NP and VP40 Real-time RT-PCRs, DoDEZ1 Real time RT-PCR, RealStar (R) Ebola virus RT-PCR kit 1.0, LightMix (R) Ebola Zaire rRT-PCR and BioFire Defense LLC Film Array Biothreat-E and NGDS BT-E, with the use of prescribed equipment. b = Presently, the cost of an RT-PCR test is in the range of US \$ 60-200 (or Rs. 4000-12500) and can take a days time for results to become available. c = Rapid point-of-care diagnostic device to complement RT-PCR. d = Information not available. e = This 30 minutes assay is proven to be 100 times more sensitive than other tests

human nuclear genome (Carroll *et al.*, 2013; Park *et al.*, 2015). This means that EBOV, in the course of its passages between hosts, perhaps extends its host range and improves its contagiousness. Because of bulk of mutations may be of negative value (non-

neutral and non-positive) for virus multiplication, induction of mutations by use of mutagens, such as by the use of ribovirin to cause decreased production of infectious virions is being deployed as a therapeutic mechanism (Alfson *et al.*, 2016).

Table 5: The emerging, pre- and post- infection, Ebola virus disease controlling drugs and vaccines of high potential (undergoing proof of concept, preclinical or clinical trials)^a

S.No.	Drug/ vaccine	Name	Composition	Mode of action, other properties and remarks	Manufacturer	Reference(s)
1	Small molecular drug	Favipiravir (T-705 or Avigan)	Pyrazinecarboxamide derivative	Blocks RNA polymerase; an anti-avian flu drug available as tablets	Fujifilm Holdings Corp (Toyama Chemical Co Ltd)	Oestereich <i>et al.</i> (2014); Smither <i>et al.</i> (2014); Jacobs <i>et al.</i> (2015); Sissoko <i>et al.</i> (2016)
2		Brincidofavir (CMX-001)	Hexadecyloxy-propylcidofavir (a nucleotide analog, phosphorylated cytidine added with a lipid chain)	Disrupts the RNA polymerase function; a broad spectrum drug, is active against double stranded DNA cytomegalovirus, adenovirus, BK virus and herpes simplex virus; available as tablets	Chimerix Inc	Florescu and Keck (2014)
3		BCX4430 (Immucillin)	Synthetic adenosine nucleoside analogue	Incorporation in RNA causes premature termination of RNA polymerase function; is active against a range of RNA viruses; originally developed for hepatitis C	Biocryst Pharmaceuticals Inc	Warren <i>et al.</i> (2014)
4		JK-05	NR ^b	Inhibitor of RNA polymerase; efficacious against influenza and yellow fever viruses	Sihuan Pharmaceutical	Wu and Liu (2014)
5		Retinazone	Vitamin A derived thiosemicarbozone derivative	Virus multiplication is terminated by covalent activation of iatrogenic and intraaxonic viral glucocorticoid response elements; is anti HIV, Varicella-zoster, HBV, HCV and Cytomegalovirus	Aventis Pharma	Kesel <i>et al.</i> (2014)
6		Ribavirin	Guanosine analogue	Interferes with RNA polymerase function	BioCryst Pharmaceuticals Inc	Goeijenbier <i>et al.</i> (2014); Alfson <i>et al.</i> (2016)
7		GS-5734	Nucleotide analog	Interfere with viral RNA replication; is active against a broad spectrum of viral pathogens. (Middle East Respiratory Syndrome virus, Marburg virus and multiple variants of Ebola virus); given three days after infection, it completely protected rhesus monkeys from Ebola virus	US Army Medical Research Institute	Warren T <i>et al.</i> (2015a); Warren <i>et al.</i> (2016)
8		IE7-03	Tetrahydroquinolone derivative targets dephosphorylation function of protein phosphatase	Phosphorylation of VP30 is increased leading to suppression of virus multiplication	An undisclosed company	Ilinykh <i>et al.</i> (2014)
9		Oubain	Na ⁺ K ⁺ -ATPase inhibitor	Disrupts the activity of ATP1A1 and thereby VP24 activity in Ebola infected cells halting viral multiplication	Selleck Chemicals	Garcia-Doriwal <i>et al.</i> (2014)
10		Lamivudine (EpiVir)	(-)-2', 3'-dideoxy 3'-thiacytidine; a reverse transcriptase inhibitor	Viral multiplication is inhibited	Glaxo SmithKline	Cohen (2014)
11		CM-10-18	Imino-sugar- α -glucosidase inhibitor	Glycan processing enzymes are inhibited leading to misfolding and degradation of viral envelope glycoprotein and thereby reduction in viral egression	Promega	Chang <i>et al.</i> (2013a, b)

12	DZNep	3-Deazaneplanocin A	Boosts interferon production in patient by inhibiting S-adenosylhomocysteine synthesis and histone methyltransferase EZH2	Selleckchem com	Bray <i>et al.</i> (2002); Schuchman (2014)	
13	Tetrandrine	Bis-benzylquinoline alkaloid, naturally present in <i>Staphania tetrandra</i>	Blocks entry of Ebola into host cell by inhibiting the L-type and T-type calcium channels and Ca ²⁺ activated K ⁺ channel	Santa Cruz Biotechnology and Sigma-Aldrich	Bhakuni <i>et al.</i> (1980); Sakurai <i>et al.</i> (2015)	
14	Diazachry-sene	Anti-malaria and -flu drug	Blocks viral replication	DuPont De Nemours & Co.	Selakovic <i>et al.</i> (2015)	
15	Melatonin	Animal hormone N-acetyl-5-methoxy-tryptamine	Its free radical scavenging, anti-inflammatory and anti-coagulant activities limit the host's lethal proinflammatory response	Various	Tan <i>et al.</i> (2014)	
16	Mannosylated fullerene sugar balls	Water soluble glycofullerene derived from C60 core binds to lectins	They block Ebola virion entry by inhibiting DC-SIGN and other lectin dependent cell infection	Nano-C	Luczkowiak <i>et al.</i> (2013); Nierengarten and Nierengarten (2014); Munoz <i>et al.</i> (2015)	
17	V18666A	A cationic amphiphile	Induces cholesterol accumulation in endosomes and thereby blocks viral entry into cells	Experimental	Cenedella (2009); Haque <i>et al.</i> (2015); http://www.ncbi.nlm.nih.gov/pcoupound?Term=U18666A	
18	FGI-103	Replication inhibitor	Not known	As above	Warren <i>et al.</i> (2010); Haque <i>et al.</i> (2015); http://chem.sis.nlm.nih.gov/chemidplus/rn/907169-69-1	
19	KIN 1409 (and KIN1408)	A hydroxyquinoline	Activates interferon regulatory factor 3 (IRF3) and thereby expression of antiviral genes	Kineta Inc.	Pattabhi <i>et al.</i> (2015)	
20	Combinations of molecules	OSU-03012 (AR-12) + Sildenafil (Viagra)	Mixture of celecoxib derivative AR12 and phosphodiesterase 5-inhibitor viagra	Selleck Chemicals and Pfizer	Brooth <i>et al.</i> (2014); Booth <i>et al.</i> (2015)	
21	Atorvastatin + Irbesatan	Mixture of generic statin and angiotensin receptor blocker	Restores endothelial barrier integrity and stops fluid and mineral losses	Various companies	Opal <i>et al.</i> (2015)	
22	Miglustat + Toremfifene	Mixture of D-glucose analogue and cationic amphiphile	Viral multiplication is limited by inhibition of NPC1 function and blockage of viral secretion and envelope formation steps	Experimental	Mehta <i>et al.</i> (1998); Chang <i>et al.</i> (2013); Shoemaker <i>et al.</i> (2013); Yuan <i>et al.</i> (2015)	
23	Clomiphene + Toremfifene	Mixture of estrogen receptor modulators	Inhibition of virus replication by NPC1 overexpression leading to cholesterol accumulation in endosomes	As above	Johansen <i>et al.</i> (2013); Shoemaker <i>et al.</i> (2013); Haque <i>et al.</i> (2015)	
24	Extract of <i>Cistus ncanus</i> plant	Combination of secondary metabolites	Metabolites present in the extract block the attachment of viral envelope proteins to the cell surface	As above	Rebensburg <i>et al.</i> (2016)	
25	RNA drug	TKM-Ebola and siRNA-LNP-Ebola	siRNAs against RNA polymerase, VP24 and/or VP35	Inhibits viral multiplication via degradation of viral RNA and shown to combat lethal disease in Rhesus monkeys	Tekmira Pharmaceuticals	Geisbert <i>et al.</i> (2010); Thi <i>et al.</i> (2015 and 2016) and/or VP35

26	AVI-7537	Phosphorodiamidate morphino nucleotide oligomer antiparallel to VP24 AUG codon proximal mRNA segment inhibits multiple roles of VP24 in Ebola life cycle	Blocks the translation of VP24 mRNA essential for viral multiplication	Sarepta Therapeutics Inc	Iversen <i>et al.</i> (2012); Heald <i>et al.</i> (2014); Warren <i>et al.</i> (2015b)	
27	Nanoviricide synthetic ligand	Ebola binding	A polymer backbone mounted with a virus binding ligand that mimicks the Niemann-Pick C1 protein Ebola virus receptor on human cells thus acting as biomimetic anti-Ebola medication	Nanoviricide Inc	www.nanoviricide.com	
28	Protein/peptide drug	Griffithsin (from red algae <i>Griffithsia</i> species as well as transgenic <i>Nicotiana benthamiana</i>)	Mannose binding lectin	Breaks down the Ebola virion envelope by binding to N-linked glycans present on virion surface	Cayman chemical	O'Keefe <i>et al.</i> (2009); Barton <i>et al.</i> (2014)
29	F4-6	Fibrin derived peptide	Given together with antibiotics, it blocked vascular leakage in EVD patient and cured Ebola infection	MChE-F4 Pharma	Wolf <i>et al.</i> (2014)	
30	Z Mapp	Cocktail of monoclonal antibodies against three Ebola GP protein epitopes produced in transgenic <i>Nicotiana benthamiana</i> plants	Binding of Ebola virus to host cells is interfered; GP and sGP proteins are targeted: a need for substitution/addition of new Bio monoclonal antibodies against GP protein has been demonstrated; also to improve its effectivity addition of antibodies from Ebola convalescents has been considered	Mapp Biopharmaceuticals Inc, (Leaf Inc) Defyrus Inc, US Government, and Public Health Agency of Canada	Olinger <i>et al.</i> (2012); Qiu <i>et al.</i> (2012 and 2014); Murin <i>et al.</i> (2014); Kugelman <i>et al.</i> (2015); Pallesen <i>et al.</i> 2016; Spence <i>et al.</i> (2016)	
31	mAb114	Single monoclonal antibody isolated from a human EVD survivor	Three consecutive intravenous injections given as late as five days after infection completely protected non-human primates from Zaire lethal Ebola infection	Experimental	Corti <i>et al.</i> (2016); Misasi <i>et al.</i> (2016)	
32	E4 and E10 16F6 GP derivatives of murine antibody	Immunotherapeutic cocktail of synthetic monoclonal antibodies	Monoclonal antibodies against GP protein effective against Sudan Ebola Virus	US Army Medical Research Institute of Infectious Diseases	Chen <i>et al.</i> (2014)	
33	Mixture of small molecule and peptide drugs	Chloroquine+ Atorvastatin, Pyridinyl imidazole, Artemisinin, and/or FTY720 + F4-6	Antiviral cocktail for viral entrapment in endosomes and control of inflammation and coagulation	Chloroquine stops the exit of virus from endosomes, statin blocks NCP1 function, pyridinyl imidazole controls cytokine production from infected dendritic cells, artemisinin inhibits inflammation, FTY720 controls the immunopathologic response and F4-6 cures any vascular leakage	Experimental	Walsh <i>et al.</i> (2011); Fedson (2013); Madrid <i>et al.</i> (2013); Ho <i>et al.</i> (2014); Johnson <i>et al.</i> (2014); Haque <i>et al.</i> (2015); Wolf <i>et al.</i> (2015)

34	Blood or serum as drug	Convalescent whole blood or serum	Whole blood or serum collected from EVD patients cured of infection	Blood obtained from patients four weeks after disappearance of Ebola from their system is used on patients of EVD to neutralize Ebola virus components in them with antibodies present in the convalescent blood/ serum	Local physicians	WHO/HIS/SDS/2014.8
35	Vaccine	Ch Ad3/ EBOV ^a	Chimpanzee adenovirus-3 carrying the Ebola gene from Zaire- and Sudan-Ebolas	Active against ZEBOV and SEBOV virus diseases; single dose of 1×10^{11} pu serves as ring vaccination to interrupt ebola transmission and if followed 2-3 months later by a boosting dose of MVA-BN-Filo (Modified Vaccinia Ankara expressing Zaire Ebola-virus glycoprotein) provides long-lived protection	Glaxo Smith Kline	Stanley <i>et al.</i> (2014); Hoenen and Feldmann (2014); Tapia <i>et al.</i> (2016)
36		Ad-CAGopt z GP	Similar to the above recombinant vaccine but breathable	A single dose of 1.4×10^9 infectious particles/ kg induces long-lasting protection	National Institute of Health, USA	Choi <i>et al.</i> (2014)
37		VSV Δ G/ EBOV GP and Vesiculovax	Attenuated vesicular stomatitis virus integrated with GP gene of Ebola	Recombinant replication competent vesicular stomatitis virus that expresses Ebola GP protein; is shown to be effective in single dose. Macaques vaccinated a week before challenge of EBOV strains Mayinga, Kikwit and Makona were protected. Ring vaccination of human populations in Guinea effectively blocked EVD outbreak in a trial. Single dose proved to be mildly reactogenic but highly immunogenic, with no serious adverse effect on humans	New Link Genetics Corporation; Profectus Biosciences	Marzi <i>et al.</i> (2013 and 2015a,b,c); Chad <i>et al.</i> (2015); Regules <i>et al.</i> (2015); Henao-Restrepo <i>et al.</i> (2015 and 2016); Agnandji <i>et al.</i> (2016); Tapia <i>et al.</i> (2016); www.profectusbiosciences.net
38		Rabies/EBOV	Wild type, replication deficient or inactivated Rabies virus that expresses GP gene of Ebola that protects against rabies and ebola diseases	Recombinant rabies virus that expresses Ebola GP protein	Patent US20140212434 assigned to USA represented by Dept. of Health and Human Services	Blaney <i>et al.</i> (2013)
39		Ebola whole inactivated virus	Hydrogen peroxide inactivated VP30 deleted whole Ebola virus	Robust immune response elicited by all viral proteins except VP30	NIH and JHLSc (Japan), University of Wisconsin ^d	Marzi <i>et al.</i> (2015d)
40		HPIV3/ EboGP	Human parainfluenza type 3 that expresses the Ebola GP gene	Aerosolized vaccine delivered to respiratory tract is safe, immunogenic and protective; a single dose protected 100% of infected macaques	Phase 1 trial is in progress	Meyer <i>et al.</i> (2015)
41		CMV/ EBOVGP	Herpes virus called Cytomegalovirus altered to express Ebola virus glycoprotein gene GP	Vaccine provides protection to Rhesus monkeys against Ebola virus; it is self disseminating to be used also to target Ebola in non-human apes in the wild	Experimental	Murphy <i>et al.</i> (2015); Marzi <i>et al.</i> (2016)

a = Molecular structures of several of the compounds listed in this table are provided in the figures 4 and 5; b = NR, not revealed; c= Japanese Health and Labour Sciences; d = Madison

Table 6: Approved drugs found to control Ebola virus infection in repurposing screens

S.No.	Drug(s) approved by Food and Drug Administration of United States of America for use in humans and veterinary medicine	Disease condition(s) for which indicated	Mode of action	Reference(s)
1	Tilorone	Antiviral	DNA polymerase inhibition	Kouznetsova <i>et al.</i> (2014)
2	Azithromycin, Clarithromycin, Dirithromycin, Erythromycin, Spiramycin	Antibacterial	Protein synthesis inhibition	Madrid <i>et al.</i> (2013) ; Kouznetsova <i>et al.</i> (2014)
3	Maduramicin, Nitrovin	As above	Ionophore	Kouznetsova <i>et al.</i> (2014)
4	Amorolfine, Posaconazole, Terconazole	Antifungal	Membrane-bound enzyme (sterol synthesis) inhibition	Shoemaker <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014)
5	Chloroquine	Antimalarial	Inhibition of hemozoin formation	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014)
6	Amodiaquine, Mefloquin	As above	Inhibition of histamine N-methyltransferase	As above
7	Bosutinib	Anticancer	Bcr-Abl tyrosine kinase inhibition	As above
8	Carfilzomib	As above	Proteasome inhibition	As above
9	Daunomycin, Daunorubicin, Topotecan	As above	Topoisomerase inhibition	As above
10	Erlotinib, Gefitinib, Genistein	As above	Epidermal growth factor receptor inhibition	Kolokoltsov <i>et al.</i> (2012); Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014) and www.int/medicines/emp/ebola_q_as/en/
11	Imatinib, Nilotinib, Sunitinib, Tyrphostin	As above	Kinase inhibition	Garcia <i>et al.</i> (2012); Kolokoltsov <i>et al.</i> (2012); Kouznetsova <i>et al.</i> (2014); Napier <i>et al.</i> 2015
12	Relaxifene, Tamoxifene, Toremifene	As above	Estrogen receptor modulation	Shoemaker <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014)
13	Navelbine, Nocardazole, Vinblastine, Vincristine	As above	Microtubule inhibition	As above
14	Colchicine	Gout	As above	Kouznetsova <i>et al.</i> (2014)
15	Mycophenolate mofetil	Immuno-suppression	Inhibition of inosine monophosphate dehydrogenase	Madrid <i>et al.</i> (2013)
16	Albendazole, Mebendazole	Anthelmintic	Microtubule inhibition	Kouznetsova <i>et al.</i> (2014)
17	Oxibendazole	As above	DNA polymerase inhibition	As above
18	Niclosamide	As above	STAT-3 inhibition	As above
19	Cephranthine	Antineoplastic and Anti-inflammatory	Inhibition of release of neutrophil elastase	As above
20	Aprindine, Deslanoside, Digoxin	Antirhythmic	Inhibition of Na ⁺ K ⁺ pump	Gehring <i>et al.</i> (2014); Kouznetsova <i>et al.</i> (2014)
21	Amiodarone, Prepfenone	As above	Na ⁺ channel blocking	As above
22	Dronedarone	As above	Multichannel blocking	As above
23	Amlodipine, Mibefradil, Verapamil	Antihypertensive	Calcium channel blocking	Garcia <i>et al.</i> (2012); Kolokoltsov <i>et al.</i> (2012); Madrid <i>et al.</i> (2013); Shoemaker <i>et al.</i> (2013); Gehring <i>et al.</i> (2014); Kouznetsova <i>et al.</i> (2014); Napier <i>et al.</i> (2015)

Table 6: Contd....

(1)	(2)	(3)	(4)	(5)
24	Lomerizine, Bepridil	Migraine	As above	Johansen <i>et al.</i> (2015)
25	Cyclomethycaine, Dibucaine	Local anaesthetic	Na+ K+ pump inhibition	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014)
26	Levopropoxyphene	Antitussive	K+ efflux increased, Ca+ flux reduced	Madrid <i>et al.</i> (2013)
27	Bitolterol, Penbutolol, Salmeterol	Antiasthma/ Bronchodilator	Beta-adrenergic receptor antagonism	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014)
28	Dipivefrin	Glaucoma	Adrenergic antagonist	Madrid <i>et al.</i> (2013)
29	Azaclorzine	Antianginal	As above	Kouznetsova <i>et al.</i> (2014)
30	Propagenone	Ventricular arrythmas	As above	Cheng <i>et al.</i> (2015)
31	Prochlorperazine, Trifluoperazine	Antiemetic	Dopamine antagonism	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014)
32	Piperacetazine, Thiothixene	Antipsychotic	As above	Kouznetsova <i>et al.</i> (2014)
33	Dilazep	Coronary heart disease	As above	Cheng <i>et al.</i> (2015)
34	Thiopropazine	As above	Postsynaptic receptor modulation	As above
35	Carprofen, Proglumetacin	Anti-inflammatory	Cyclooxygenase-1 inhibition	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014)
36	Bezedoxifene, Clomiphene, Estradiol, Toremifene	Female fertility/ post menopaual osteoporosis	Estrogen receptor modulation	Johansen <i>et al.</i> (2013); Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014); Rhein and Maury (2015)
37	Alendronate	Osteoporosis	Sterol synthesis inhibition	Shoemaker <i>et al.</i> (2013)
38	Alverine citrate	Antispasmodic	Modulation of parasympathetic nervous system	Kouznetsova <i>et al.</i> (2014)
39	Clemastine, Cyproheptadine, Dexbrompheniramine, Ketotifen	Antiallergic/ Hay fever/ Rhinitis	Histamine antagonism	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014); Cheng <i>et al.</i> (2015)
40	Aripiprazole	Antipsychotic	Antagonist of 5-HT-1A and -2A receptors	Johansen <i>et al.</i> (2015)
41	Benztropine, Biperiden, Diphenylpyraline, Oxyphencyclimine, Trihexyphenidyl	Antihistamine and/or Antiparkinson	Histamine and cholinergic antagonism	As above
42	Diphenoxylate	Antiperistaltic	Opiate receptor antagonism	Madrid <i>et al.</i> (2013)
43	Clomipramine, Trimipramine	Antidepressant	Histamine antagonism and serotonin uptake inhibition	Miller <i>et al.</i> (2012a); Kouznetsova <i>et al.</i> (2014)
44	Maprotiline	As above	Histamine antagonism and adrenergic uptake inhibition	Kouznetsova <i>et al.</i> (2014)
45	Fluoxetine, Sertraline	As above	Serotonin uptake inhibition	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014); Johansen <i>et al.</i> (2015)
46	Bifemelane	As above	Cholinergic system modulation	Kouznetsova <i>et al.</i> (2014)
47	Imipramine, Desipramine, Paraxetine, Protrityline	As above	Serotonin norpinephrine reuptake inhibition	Madrid <i>et al.</i> (2013); Kouznetsova <i>et al.</i> (2014); Rhein and Maury (2015)
48	Promethazine	Antiallergic	Muscarinic acetocholine receptor antagonist	Cheng <i>et al.</i> (2015)
49	Gamma-interferon	Chronic granulomatous disease and osteoporosis	Blocks infection of macrophages and dendritic cells	Rhein <i>et al.</i> (2015)

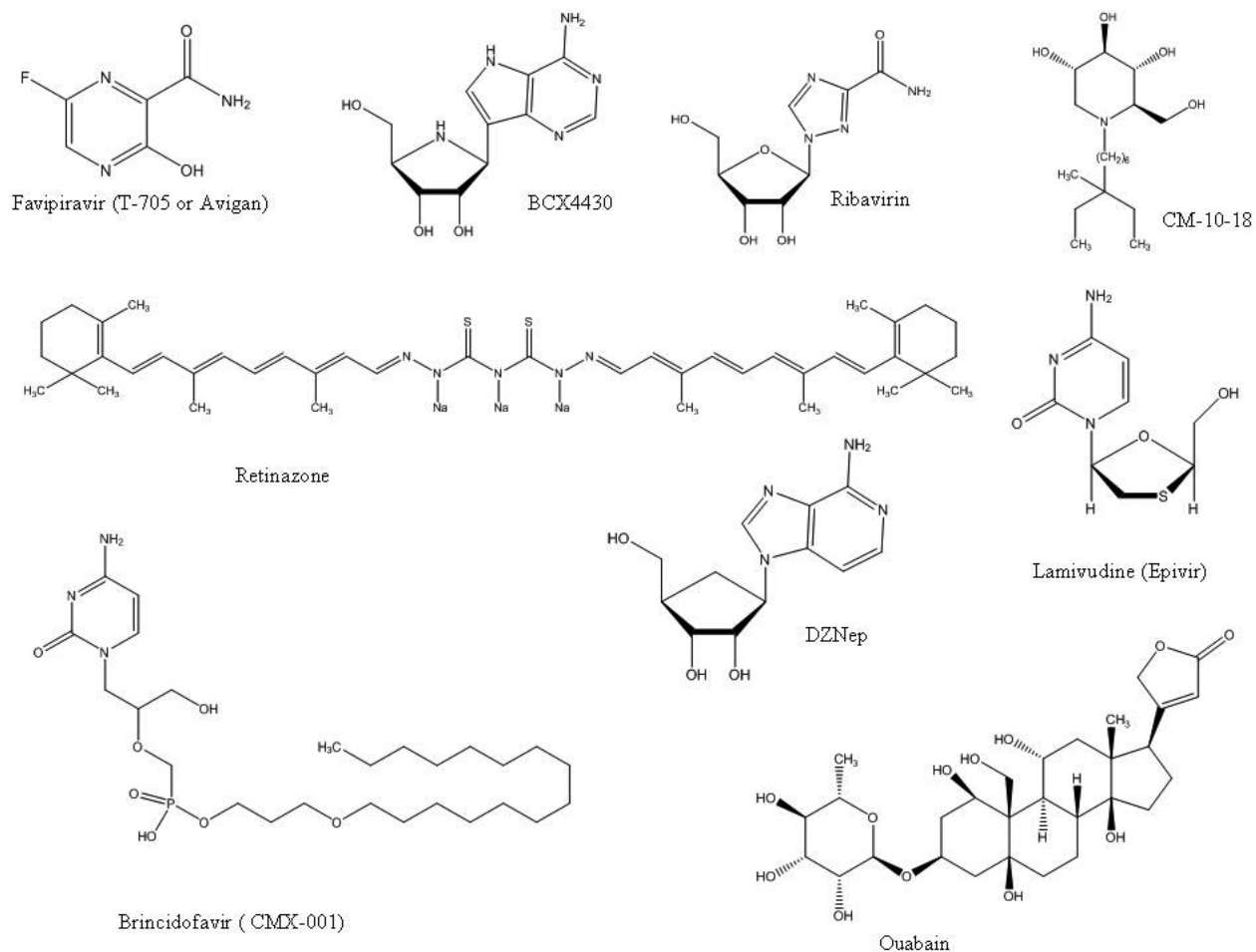


Fig. 4: Molecular structures of Favipiravir, Bcx4430, Ribavirin, CM-10-18, Retinazone, DZNep, Lamivudine, Brincidofovir and Oubain, compounds under development as Ebola virus disease treatments and medicines. The properties of the compounds are summarized in the Table 5

Analysis of 81 Ebola genome sequences of origin in the recent outbreak in Sierra Leone (West Africa) revealed 341 mutations that were new and not present in previously sequenced EBOVs of origin in central Africa. L, NP and VP24 were the genes in which a majority of mutations were localized (Gire *et al.*, 2014). Mutation A-82V in GP gene increased Ebola's infectivity towards human cells highly significantly and thus achieved greater adaptability for humans (Diehl *et al.*, 2016).

A forward genetics experiment has shown that non-synonymous substitution mutations in the EBOV GP gene increased Ebola's human cell tropism and decreased tropism towards bat cells, suggesting evolution of increased infectivity against humans in Ebola (Urabanowicz *et al.*, 2016).

Ebola undergoes mutations not only during

animal-to-human and human-to-human transmission, but also during animal-to-animal transmission. EBOV infection in guinea pigs is asymptomatic; infected animals multiply the virus, but are devoid of clinical symptoms. Five and seven time passaging of Ebola in guinea pigs, respectively, led to the development of clinical symptoms and death. Mutations in GP, NP, VP35, VP24 and/or L and not in VP30 and VP40 were observed to be responsible for increase in virulence by repeat passaging of virus in guinea pigs. Significantly, virulence increase was associated with increased rate of editing in GP and mutation at position 26 in VP24 (Subbotina *et al.*, 2010; Dowell *et al.*, 2014). It is thought that in future when vaccines and treatments against Ebola become available, EBOV may survive by evolving decreased virulence and increased contagiousness.

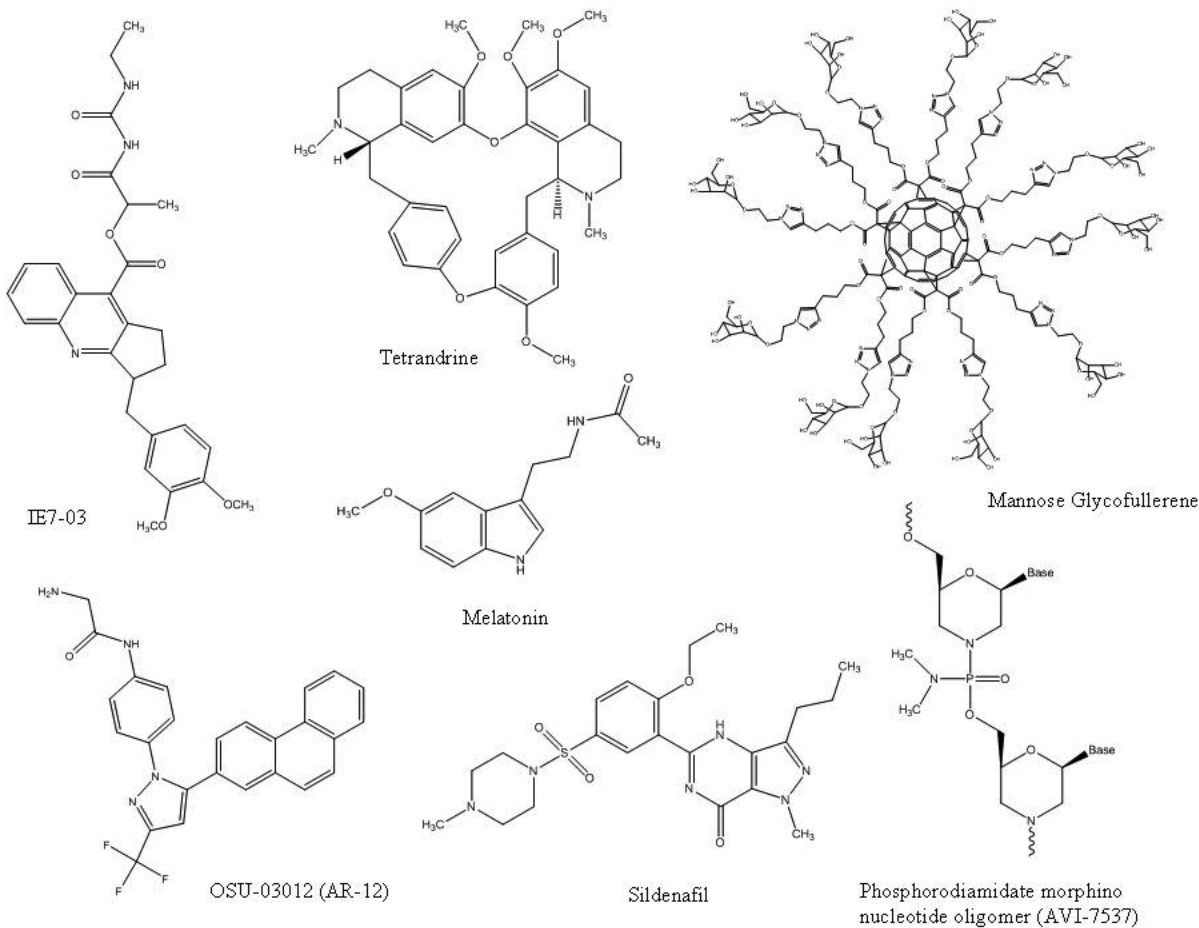


Fig. 5: Molecular structures of IE7-03, Tetrandrine, Melatonin, OSU-03012 (AR-12), Sildenafil, Mannosylated glycofullerene and Phosphorodiamidate morphinonucleotide oligomer (AVI-7537), compounds under development as Ebola virus disease treatments and medicines. The properties of the compounds are summarized in the Table 5

Ebola Virus x Host Interactions

Biology of Virus Entry Into and Multiplication in Host Cells

Ebola virus present in secretions and excretions of infected animal/human Ebola-diseased enters human host via the mucosal surfaces (in eyelids, lips of mouth, nostrils, ears, anus and man and woman genitals) and injured skin. Dendritic cells, macrophages and monocytes (of the mononuclear phagocytic system) are the initial targets of Ebola virus entry and for its multiplication. Subsequently, multiple mechanisms, including migration of infected mononuclear phagocytic cells and release of virions into lymphoid system or blood stream, make the infection systemic. Besides the macrophages, monocytes and dendritic cells, hepatocytes, adrenal cortical cells, fibroblasts, endothelial and epithelial cells of various tissues and

organs of host body get infected and fatal infection results from necrosis of infected cells (Mahanty and Bray 2004; Feldmann and Geisbert 2011; Olejnik *et al.*, 2011; Chiappelli *et al.*, 2015; de La Vega *et al.*, 2015).

Ebola virus replication cycle is shown diagrammatically in the Figure 2C. To gain entry into host cell, virion attaches to the receptors present on the surface of the susceptible host cell via its glycoprotein GP1 present on virus envelope. The interaction of phosphatidylserine present on cell membrane and Ebola virion facilitates this process. Host cells demonstrate differences in the spectrum of Ebola receptors present on their surface. Some of the receptors are: folate receptor- α , C-type lectin family proteins such as DC-SIGN, L-SIGN, L SECT, hMGL, and asialo-glycoprotein, T-cell immunoglobulin

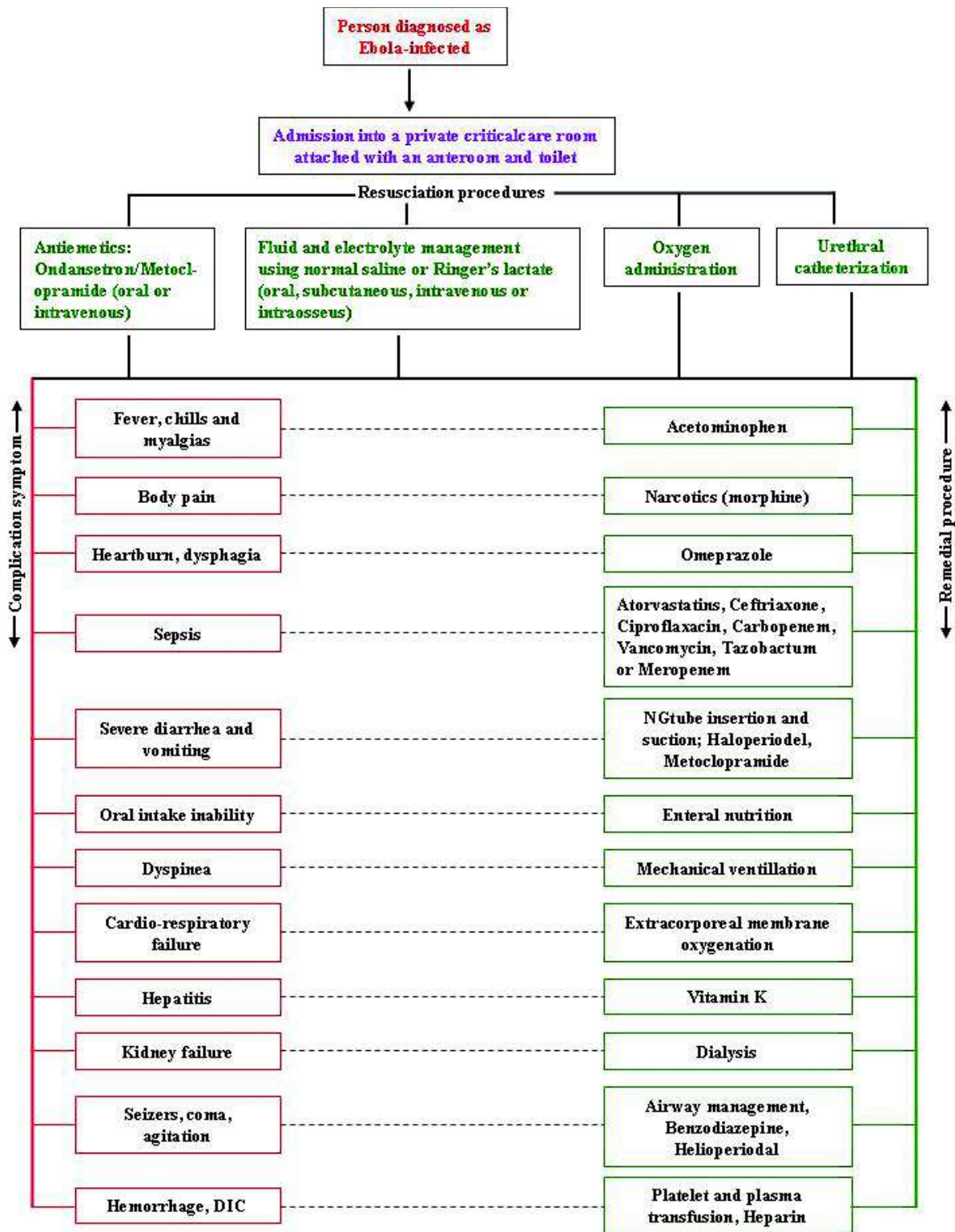


Fig. 6: Supportive care and symptomatic treatment of complications of Ebola virus disease are diagrammatically presented. The diagramme interventions, initiated as early as possible, significantly improved survival of Ebola-infected persons. The basic interventions are providing fluids and maintaining electrolyte balance, oxygen status and blood pressure. Complications are treated as they appear (Kiraly *et al.*, 2006; Levi *et al.*, 2010; Walley *et al.*, 2011; Clark *et al.*, 2012; Patel *et al.*, 2012; Yonus *et al.*, 2012; Fedson 2014; Roberts and Perner 2014; Funk and Kumar 2014 and 2015; Anonymous 2015; WHO.H422128455; Chiappelli *et al.*, 2015; Ker *et al.*, 2015). With the gain of experience and approval of clinical vaccine and therapies, presently under trial, the supportive care will get converted into treatment, in near future

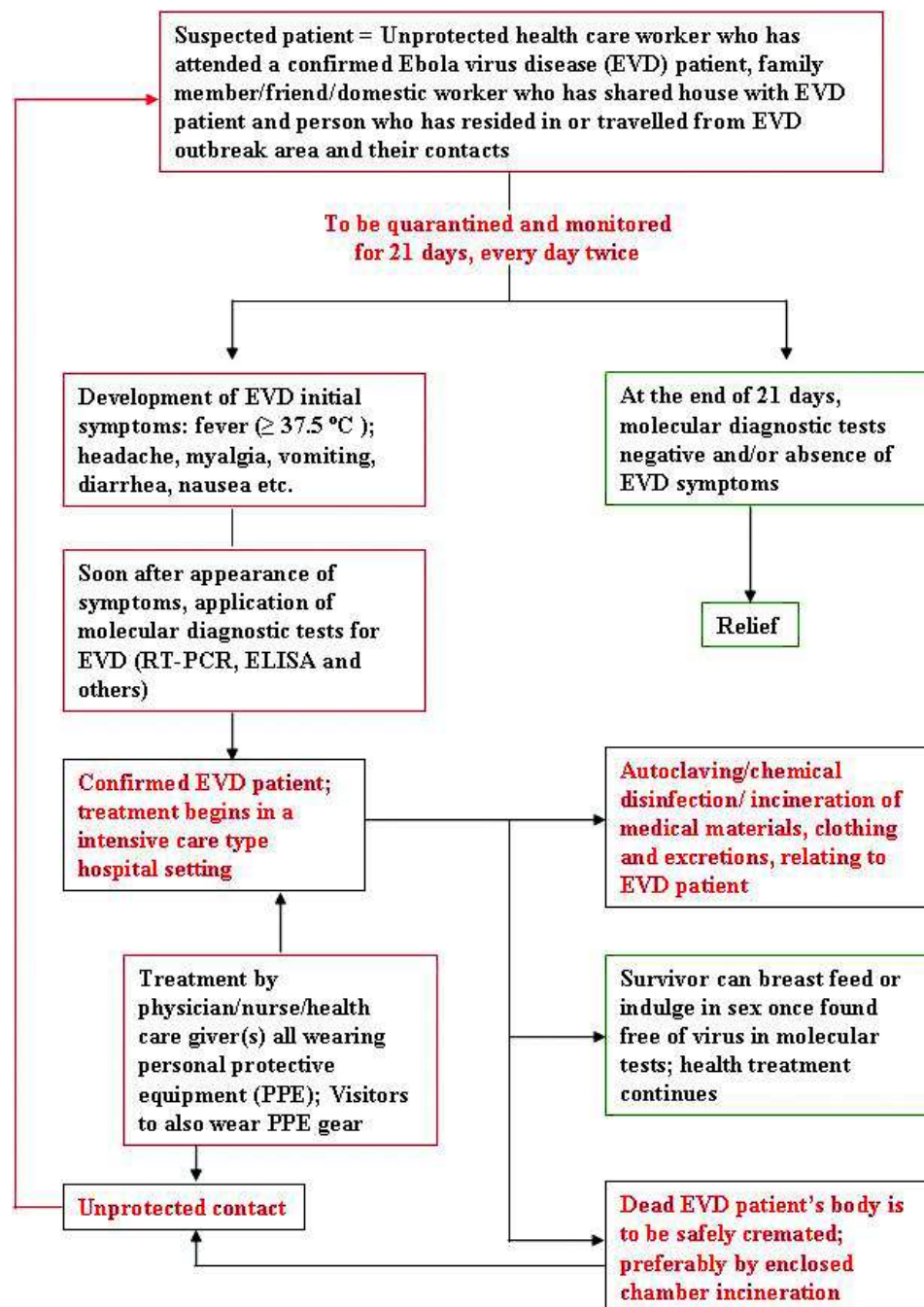


Fig. 7: Diagrammatic representation of the pathway to contain spread of Ebola virus disease (EVD), infection/transmission in an affected locality/African country, and, to stop import of EVD infection into a new country/different continent

mucin domain (TIM-1) protein, Tyro3/Axl/Mer (TAM) family proteins, integrin β , G protein coupled receptors, etc (Volchkov *et al.*, 1998; Weissenhorn *et al.*, 1998; Chan *et al.*, 2001; Takada *et al.*, 2003; Marzi *et al.*, 2006a and 2006b; Hunt *et al.*, 2011; Lennemann *et al.*, 2014; Cheng *et al.*, 2015; Rhein and Maury 2015).

Following attachment, virion gets enclosed into

a ruffle/bleb (macropinosome) of plasma membrane. Macropinocytosis internalizes the macropinosome (Hunt *et al.*, 2011), which is then trafficked to the endolysosomal pathway (Bhattacharyya *et al.*, 2012; Miller and Chandran 2012; Nanbo *et al.*, 2010; Saeed *et al.*, 2010; Carette *et al.*, 2011). Under the acidic environment of endosome, cysteine proteases cathepsin-B and -L and other proteases cleave GP1

such that its aminoterminal domain gets exposed, which is ligand for the intracellular receptor Niemann-Pick Type C1 (NPC1 = Cholesterol transporter) protein of host plasma membrane. NPC1 and GP1 interaction is essential for the viral and host plasma membrane fusion (Carette *et al.*, 2011; Cote *et al.*, 2011; Miller and Chandran 2012; Herbert *et al.*, 2015; Spence *et al.*, 2016). Conformational changes in GP2 (a class I viral fusion protein) unmask its fusogenic domain (45aa loop near to N-terminus) and also such domains in the endosomal membrane such that fusion occurs between them on their interaction (Chandran *et al.*, 2005; Hood *et al.*, 2010; Gregory *et al.*, 2011; Miller and Chandran 2012). As a consequence the ribonucleocapsid of virion is released into host cell cytoplasm.

In the host cell cytoplasm, the virus genome which is a part of somewhat relaxed ribonucleocapsid, serves as a template for the sequential transcription of all the seven genes, into monocistronic mRNAs, which are capped and polyadenylated and translated to produce viral proteins. When enough of NP has been produced, genome replication starts. Nascent +ive (antigenome) and -ive (genome) RNAs assemble into their nucleocapsids. VP35 chaperones NP to replication complex, helps NP to coil around the progeny RNA and form the nucleocapsid shell (Kirchdoerfer *et al.*, 2015; Leung *et al.*, 2015). In the self assembly process of encapsidation, RNA-NP interaction makes RNA helicoidal and allows interaction with VP30, VP35 and L proteins (Watanabe *et al.*, 2006 and 2007; Bharat *et al.*, 2012). Termination of the replication phase and onset of virion assembly and egress phase is controlled by VP24. Virus budding and egress involves the viral proteins VP24, VP40 and GP and host protein machinery, including endosomal sorting complex required for transport (ESCRT) (Han *et al.*, 2003; Jasenosky and Kawaoka 2004; Licata *et al.*, 2004; Watanabe *et al.*, 2007). Nucleocapsids arrive at VP40 and GP containing multivesicular bodies at the plasma membrane for their constitution as virions and egress (Dolnik *et al.*, 2004; Hartlieb and Weissenhorn 2006; Liu *et al.*, 2010). Virus particle formation is aided by phosphatidyl serine at the plasma membrane (Soni *et al.*, 2013; Soni and Stahelin 2014). Egress is accompanied by virus particles acquiring GP studded cellular plasma membrane lipid bilayer linked to ribonucleocapsid by the matrix proteins VP24 and

VP40 (Klenk and Feldmann 2004; Chandran *et al.*, 2005; Manicassamy *et al.*, 2005; Marzi *et al.*, 2006a, b; Hoenen *et al.*, 2010; Liu *et al.*, 2010; Olejnik *et al.*, 2011; Adu-Gyamfi *et al.*, 2013).

The variation in the incubation period of 2 to 21/23 days, before symptoms of Ebola disease development start to appear, is thought to depend on the size and route of virus inoculum and host genetic factors that determine the degree of success in mounting the innate adaptive response against the virus multiplication. In the survivors of Ebola virus disease, the viral titer from initial stages onwards is 100 to 1000 fold lower than non-survivors. The patients nearing death may have 10 billion virus particles in their body.

In patients recovering from EVD, replicating viral RNAs are present in lower and upper respiratory tracts for several to many days, after RNA is no longer detectable in blood plasma (Biava *et al.*, 2017). This suggests that though the tissues of all body organs get infected, there is major role of lung tissues in pathogenesis and raises the possibility of transmission via exhaled air.

Host Pathophysiology

Highly virulent property of Ebolavirus is responsible for the development of severely sick pathophysiology of deadly consequences in humans. Pathophysiology of fatal EVD involves more or less the entire body of the patient and several aspects of it remain to be understood. Much of the information about EVD pathogenesis has been gained from case reports of the diseased persons of the previous and current EVD outbreaks and laboratory studies on animal model systems. Some of the important turning points in the progression of fatal EVD pathogenesis are: Rapid replication of Ebola virus and correlated impairment of host's innate and adaptive antiviral immune response(s); Invasion of body organs via lymph and blood stream and cellular/tissue necrosis in them; Host's pro-inflammatory response to virus/cellular debris; and collapse of vascular system, hemorrhaging, multi-organ failure, drop in blood pressure, hypovolemic shock and death. These broad features of the human EVD pathophysiology are diagrammed in Fig. 3 and aspect-wise discussed below in some detail.

Innate Immune Response and Its Blockage by Ebolavirus

Interferons are the potentiators of innate immune response. In all types of Ebola infected cells, including macrophages, monocytes and dendritic cells where infection initiates, presence of virus is sensed by the immune system receptors. Presence of Ebola activates both the cytoplasmic receptors, such as; Retinoic acid inducible (RIG)-1 and Melanoma differentiation associated (MDA)-5 and extracytoplasmic receptors exemplified by Toll-like receptor (TLR)-3, 7, 8 and 9, altogether termed as RIG-1 like receptors (RLRs). In this cascade, RLRs activate kinases-Tank binding kinase (TBK)-1 and I-kappa- B kinase epsilon (IKKE). Next, TBK-1 and IKKE phosphorylate Interferon regulatory factor (IRF)-3 and IRF-7. Thereafter, IFRs dimerize, transport to nucleus and induce transcription of Type 1 interferons (IFN) (Prins *et al.*, 2009; Yoneyama and Fujita 2009; Baum and Garcia-Sastre 2011; Kuhl and Pohlmann 2012; Garcia-Dorival *et al.*, 2014; Liu *et al.*, 2015). Secreted type 1 IFNs then bind to IFN alpha receptor (IFNAR)-1 and IFNAR-2 subunits of IFN receptor. This triggers the Janus kinase (JAK) and Signal transducer and activator of transcription (STAT) signaling cascade. Auto-phosphorylated JAK-1 and Tyrosine kinase (TYK)-3 phosphorylate STAT-1 and STAT-2. STAT-1 homodimers and STAT-1/STAT-2 heterodimers get transported to nucleus. Nuclear transport of STAT is enabled by interaction with Karyopherin (KPN)-alpha (nuclear transporters). STATs induce the transcription of IFN stimulated genes (ISG). Among the IGS products are included Interferon inducible transmembrane (IFITM) protein and Tethrin, which respectively block entry of Ebola virion into host cell cytoplasm via interaction with NCP-1 and stop Ebola virion budding by interaction with host cell plasma membrane (Schindler *et al.*, 2007; Sadler and William 2008; Schoggins *et al.*, 2011). The other ISGs, that allow establishment of anti-Ebola state, synthesized are double strand RNA dependent Protein kinase (PK)-R (PKR), its oligo adenylate synthetase (OAs), RNase L, RNA specific adenosine deaminase, and Major histocompatibility (MHC) class 1 and 2 proteins.

Ebola virus deploys its proteins VP35, VP24 and GP1,2 to blunt the IFN mediated innate immune response in multiple ways (Kimberlin *et al.*, 2010;

Leroy *et al.*, 2011a, b; Ramanan *et al.*, 2011; Kuhl and Pohlmann 2012; Messaoudi *et al.*, 2015). VP35 blocks IFN production by suppressing RLR activation, inhibition of IRF-3 phosphorylation and sumoylation of IRF-7 (Basler *et al.*, 2003; Basler and Palse 2004; Cardenas *et al.*, 2006; Chang *et al.*, 2009; Prins *et al.*, 2009; Luthra *et al.*, 2013). VP24 blocks ISGs expression by blocking nuclear transport of STAT-1 (Reid *et al.*, 2006; Dolnik *et al.*, 2008; Mateo *et al.*, 2009 and 2011; Daugherty and Malik 2014; van Hook 2014; Xu *et al.*, 2014). GP2 of GP1, 2 mislocalizes tethrin in plasma membrane such that tethrin cannot interfere with the VP40 based release of Ebola virions (Kaletsky *et al.*, 2009; Radoshitzky *et al.*, 2010 and 2011; Kuhl and Phlmann 2012; Audit and Kobinger 2014).

Adaptive Immune Response and its Abrogation by Ebola

White blood cells in the form of neutrophils and lymphocytes- Natural killer (NK) cells, B-cells and T-cells- comprise the components of adaptive immune response. Dendritic cell (DC) functions bridge the innate and adaptive immune systems. Presentation of pathogen antigens by DCs to T-cells starts a cascade that activates T-cell transcription factors NFAT (Nuclear factor of activated T cells) and AP (Activating protein)-1. NFAT and AP-1 induce transcription of anti-pathogen proteins in T-cells. However, Ebola infected DCs are deficient in performing their T-cell activating function. This leads to T-cell apoptosis (Reid *et al.*, 2007; Hartman *et al.*, 2008a and b; Jin *et al.*, 2010; Daugherty and Malik 2014; van Hook 2014; Xu *et al.*, 2014). sGP disables neutrophils; the absence of neutrophil function allows extensive viral replication (Leroy *et al.*, 2011a and b; Mohan *et al.*, 2012). sGP soaks any anti-Ebola (anti-GP1.2) IgG and IgM antibodies synthesized by B-cells and arrests movement of B-cells. Altogether, host's adaptive immune system gets compromised and Ebola is able to invade body organs (Geisbert *et al.*, 2000 and 2003a, b; Baize *et al.*, 2002; Hartman *et al.*, 2008; Jin *et al.*, 2010; Leroy *et al.*, 2011a and b; Chen *et al.*, 2014).

Additional Cytoplasmic Effects of Ebola Virus Proteins

Besides suppressing host's immune system, Ebola proteins impair the host by several additional means.

VP35 interacts with the enzymes of SUMOylation such as SUMO F₂ enzyme Ubc 9 and appropriates the SUMOylation system (Chang *et al.*, 2009). VP35 protects the cellular translational machinery from shut-down by the action of IFN and double-stranded RNA activated Protein kinase (PK)-R (PKR) (Williams 1999; Feng *et al.*, 2007; Schumann *et al.*, 2009; Escudero-Perez *et al.*, 2014; Shuchman 2014). VP35 together with VP30 and VP40 suppresses the RNAi silencing mechanism, by interacting with TRBT (Trans-activation response RNA binding) and PACT (PKR activating) proteins that are parts of the host's RISC (RNA induced silencing complex) system (Haasnoot *et al.*, 2007; Fabozzi *et al.*, 2011). GP causes deficiency of integrins and thereby, potentiates breakdown of extracellular/intercellular matrix which leads to injury to blood vessels, consequently leading to the damage of organs (Francica *et al.*, 2010). GP bound macrophages and DCs produce/ release soluble proteins/ tissue factor(s) that immuno-modulate cell/ tissue expression, which in turn causes dysfunctional bleeding and clotting (Ansari 2014; Goeijenbier *et al.*, 2014; Burd 2015; Chiappelli *et al.*, 2015). GP1.2 expression causes many host miRNAs to be differentially expressed and several of them mediate the host cell damage (Sheng *et al.*, 2014).

Host's Pro-Inflammatory Response to Ebolavirus Multiplication Results in Failure of Organ Functions and Death

Macrophages (developed monocytic white blood cells) are present in tissues of most human body organs (Gordon *et al.*, 2014). Initially, Ebolavirus multiplies in DCs, monocytes and macrophages at the place of infection. Subsequently, movement of infected macrophages, via blood stream, spreads the infection to sister cells in various organs. From there, Ebolavirus infection gets distributed to cells of other tissues co-located with macrophages in different organs. While systemic infection through macrophages is advancing and Ebolavirus titer is rising, host cells express an aberrant pro-inflammatory response that has mortal consequence. The process leads to abundant release of chemokine proteins and reactive oxygen and nitrogen species. The kinds of cytokines produced are: Interferons, Interlukin (IL)-1 β and several other ILs, Tumor necrosis factor (TNF)-alpha, CCL4 (a macrophage inflammatory protein), CCL3 and CXCL10 (a IFN- γ induced protein), chemotactic

proteins such as Eotaxin and MCP (Monocyte chemotactic protein)-1 and growth related Oncogene-alpha (Ksiazek *et al.*, 1999; Villinger *et al.*, 1999; Geisbert *et al.*, 2003; Mahanty *et al.*, 2003; Mahanty and Bray 2004; Sanchez *et al.*, 2004; Wauquier *et al.*, 2009 and 2010; Ansari 2014; Burd 2015). This cytokine storm results in a variety of pathogenic effects. Necrosis occurs in liver, spleen, lymph nodes, thymus, pancreas, kidneys, lungs and gonads and there is extensive fluid loss. Hepatocellular necrosis leads to deficiency of coagulation and other plasma proteins. There is breakdown of endothelial barrier, leakage of blood into tissues, massive hemorrhage and drop in blood pressure. Arterioles develop small blood clots (disseminated intravascular coagulation, DIC). These, together with induction of general coagulopathy contribute to thrombocytopenia. Death follows development of hypovolemic shock syndrome (Mahanty *et al.*, 2003; Mahanty and Bray 2004; Reed *et al.*, 2004; Ruf 2004; Sanchez *et al.*, 2004; Wauquier *et al.*, 2009 and 2010; Kuhl and Pohlmann 2012; Fletcher *et al.*, 2014).

Course of Ebola-pathogenesis in Survivors

In the various outbreaks of EVD recorded in Central and Western Africa, about 25 to 75 % of the patients have been observed to survive the Ebolavirus infection. Precise knowledge as to why and how some EVD patients survive remains to be known. It is thought that the outcome of EVD may depend on the (a) genotype of the virus, (b) type, number and location of initially infected cells, (c) previously acquired immunity to Ebola or heterologous virus(es), and (d) genetic tolerance/ resistance to Ebola, in the infected person. There is evidence that survivors (a) possess activated B- and T-cells and virus specific antibodies, despite lymphopenia, (b) generate much diluted pro-inflammatory response, and therefore, occurrence of necrosis in their organs is limited/ avoided, and (c) do not suffer significant vascular damage (Ruf 2004; Wauquier *et al.*, 2009 and 2010; Fletcher *et al.*, 2014; McElroy *et al.*, 2015; Chiappelli *et al.*, 2015). Detailed knowledge about factors that lead to survival against EVD is required to develop therapies against the disease.

A deep understanding of Ebola disease pathogenesis in terms of the interactions between genes/proteins of Ebola with those of the human host

is required for designing and constructing the most logical pre- and post-recombinant vectored vaccines and for discovering effective therapeutics, especially those that do not require parenteral administration.

Host Alleles that Protect Against Ebola Disease

In the course of Ebola outbreaks in Central and Western Africa, a general observation is that people responded to Ebola differentially. Whereas many developed severe symptoms and died, some showed symptoms of the disease but survived and a third group resisted the disease completely (Ascenzi *et al.*, 2008; Leroy *et al.*, 2011a, b; Chang *et al.*, 2013a, b; Burd 2015). This indicated that there may be some human gene/s allele(s) that made people resistant to Ebola infection/disease. Mutant forms of human genes *HLA-B* (*Human Leukocyte Antigen-B*) called *Bx07* and *Bx14* have been observed to render their bearers survive the Ebola infection. Contrari-wise the alleles *Bx67* and *Bx15* make their bearers susceptible to Ebola infection leading to their death. It has been shown that the NP and VP35 proteins of the virus bind tightly to *Bx07* gene product as compared to *Bx67* protein (Sanchez *et al.*, 2007). Thus, there may be depletion of the functional NP and VP35 proteins in *Bx07 Bx07* persons.

Skin cells from *Niemann-Pick C1 (NPC1)* gene mutant human homozygotes were observed to be resistant to *in vitro* Ebola virus infection (Carette *et al.*, 2011; Cote *et al.*, 2011; Bellan *et al.*, 2014). NPC1 non-function causes in the mutants a neurodegenerative disorder due to defect in cholesterol transport and related functions. It is known that mitochondria have intimate interaction with nuclear encoded NPC1. This indicates that there could be human mitochondrial haplotypes who may tolerate Ebola infection (Bellan *et al.*, 2014). Mice mutated in their *NPC1* gene have been observed to resist Ebola infection (Cote *et al.*, 2011; Herbert *et al.*, 2015; Ng *et al.*, 2015).

A study related with response of Ebola infection in recombinant inbred lines of mice revealed that mice lines were of three types, those that resisted Ebola, another group that were susceptible to Ebola leading to death in mice and a group in which Ebola infection was largely asymptomatic. Among the resistant mice, alleles of *TIE1* and *TEK* genes were present such that the mice bearing them had a relatively more active

coagulation pathway due to better regulation of fluid passage in blood vessels (Rasmussen *et al.*, 2014).

Discovery of Ebola resisting genes and mechanism of their action will be greatly valuable in the design of vaccines and discovery of new therapeutics. For example, an inhibitor of NPC1 has been discovered (Cote *et al.*, 2011).

Symptoms and Diagnosis of Ebola Virus Disease

Common Symptoms

The incubation period (or the time period from infection to appearance of symptoms) of Ebola infection in humans is 2 to 21 days, but it could be longer (Haas 2014). Usually, EVD symptoms begin to appear 5-10 days after infection. The symptoms are mild in the beginning, but they rapidly become severe and upto 90% of the patients can die 7-10 days after the onset of symptoms. A list of symptoms that may progressively appear in the EVD patients and corresponding laboratory test indications are shown in Table 3. The first symptoms are non-specific and are similar to those of dysentery and influenza, lassa, malaria, meningitis or typhoid fever. They consist of fever (39-40 degree Celsius), fatigue, headache, nausea, soar throat and abdominal/muscle pain. Subsequently a variety of problems appear, including vomiting and diarrhea (that cause acute volume depletion and hypotension), cough, shortness of breath and chest pain, prostration, oedema, confusion and delirium. These are followed by multiple organ failure. Death is preceded by hemorrhages (Fig. 1B), mucosal hemorrhages and visceral hemorrhage effusions-diffuse coagulopathy, convulsions and shock. Laboratory findings in the initial stages show low white blood cell count and in later stages, low platelet count (50-100,000/microliter), high levels of liver enzymes (two to three times of normal) and split fibrin products (Feldmann and Geisbert 2011; Kortepeter *et al.*, 2011; Hunt 2014; Hunt *et al.*, 2011). Ebola virus infections can also be mild or asymptomatic. Elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the proportion AST : ALT :: 15 : 1 on days 6 to 8 from appearance of symptoms indicates fatal EVD. On the other hand AST : ALT :: 5 : 1 is indicative of survival (Rollin *et al.*, 2007; Kortepeter *et al.*, 2011; Hunt 2014; Hunt *et al.*, 2011). Severe diarrhoea related hypoperfusion produced metabolic acidosis and hypokalemia in patients with

fatal EVD can lead to serum levels as low as 2 m Eq. L⁻¹ (Fowler *et al.*, 2014). Some characteristics of convalescing patients are also summarized in the Table 3. Survivors of EVD often suffer from long term physical and mental health complications, chronic pancreatitis, colicky abdominal pain, such as musculoskeletal pain, tremors, memory loss, depression, headaches, tinnitus, hearing loss uveitis, photophobia, conjunctivitis and loss of eye sight, arising from damage to brain, eyes and joints, body parts poorly accessed by immune system (Scott *et al.*, 2016; Vetter *et al.*, 2016).

Diagnosis

Clinical diagnosis of Ebola infection is safely and most reliably accomplished by the use of ready-made kits of standardized reverse transcriptase polymerase chain reaction (RT-PCR), which detects the presence Ebola RNA in blood or plasma and by using enzyme linked immunosorbent assay (ELISA), which detects specific viral protein/antigen in serum, plasma or whole blood (Table 4). Biosafety level 4 conditions are used while performing the tests. The tests are performed on persons who have a history of presence among EVD patients or in the area of outbreak in preceding 23 days, within 0-2 days of the appearance of initial symptoms, especially rise in body temperature. False positive or negative results are avoided by conduct of ELISA or other test(s) and RT-PCR tests on the suspected patients of EVD. RT-PCR of urine and breast milk of lactating mothers and semen of men recovering from Ebola infection has been recommended (Bausch *et al.*, 2007; Moreau *et al.*, 2015). New automated RT-PCR blood tests, quick and easy to perform have proved accurate in field applications (Semper *et al.*, 2016; Ahrberg *et al.*, 2016). A 37 minutes test detects Ebola RNA load from finger prick in diseased and semen, breast milk and eye fluids in post-recovery patients (Ahrberg *et al.*, 2016).

Recently, three rapid point-of-care tests have been described, which complement the RT-PCR test (Table 4). The dipstick immunoassay is performed by placing a finger-pricked drop of blood on a paper strip which detects in the blood presence of VP40 matrix protein of Ebola virus as a band. The test has been shown to be 100% sensitive and 92% specific, using RT-PCR test as the standard. The kit needs to be

maintained at 4°C, otherwise no other external instrumentation is necessary (Broadhurst *et al.*, 2015). Another strip-test involves magnetic nanoparticle-based immunochromatography. The reaction of antibody against glycoprotein GP of Ebolavirus, that is coated on the paper probe, with antigen present in the blood sample is visualized as colour change on the strip (Duan *et al.*, 2015). A colour coded paper strip diagnosis based on multiplex lateral flow technology simultaneously detects Ebola, Yellow fever and Dengue virus infection(s) in persons having fever (initial symptoms of Ebola and other diseases). In this test, yet to be commercialized, antibodies to viruses are conjugated to silver nanoparticles of different sizes and electrostatically absorbed to paper in different regions of paper strip. Upon application of test serum, virus-wise antigen-antibody (NSI protein of Yellow fever and Dengue viruses and GP of Ebola virus) interaction(s) allow development of red colour for Ebola, orange for Yellow fever and green for Dengue, in their respective areas on paper strip. This 10 min test will cost as much as a pregnancy test (~Rs. 150), after which it is designed (Yen *et al.*, 2015). Recently, Cai *et al.*, (2015) have reported an optofluidic analysis system that detects presence of 0.2 plaque forming units/mL in finger pricked blood sample in under ten minutes at point of care. This test is as sensitive as the RT-PCR, the Gold standard test for EVD.

Several point-of-care affordable diagnostic tests based on metagenomic analysis of virus genomes present in samples such as blood, plasma, body tissue, nasal excretions and or stool are in offing. ViroCap and VirCapSeq-VERT are highly sensitive virome capture procedures, based on metagenomic shotgun sequencing approach, that have been respectively described by Wylie *et al.* (2015) and Briess *et al.* (2015). These procedures have both diagnostic and research applications. Besides identifying the presence of any of the 207 viruses of 34 families of RNA and DNA viruses known to infect vertebrate animals and humans, the tests detect known and unknown variants/ subspecies of viruses, without prior knowledge about their presence in clinical samples. The present cost of such a diagnostic test is about US\$40 (~Rs. 2500). Greninger *et al.* (2015) have reported a point-of-care test, based on nanopore sequencing of viral genome present in high viral titer samples of blood, plasma or tissue from patients combined with MetaPORE realtime bioinformatics

analysis, whose results become available in about 6h. Faye *et al.* (2015) have developed a mobile kit that detects ebola RNA in oral swabs of infected persons in 30 min under high temperature environment of 42°C using a rapid recombinase polymerase amplification.

A transcriptomic diagnostic blood test has been developed by Liu *et al.* (2017) which predicts whether patients with Ebola virus disease will survive or die. In this test, abundant accumulation of human mRNAs from internal organs such as liver is indicative of death and increased accumulation of transcripts from NK (natural killer) cells is predictive of survival.

Briefly, a fairly good progress is being made in developing reliable and affordable diagnosis tests for Ebola virus disease, which can be used in the field as well as in hospital laboratories.

Sterilization of Materials Contaminated with Ebola Virus

Ebolavirus retains its infectivity at room temperature for several days. To stop spread of Ebola infection, it is important to sterilize surfaces and materials contaminated with Ebola (Ebola containing exudates from EVD patients). Ultra violet light (UV) inactivates Ebola virions. UV-emitting tubes, lamps and torches are available to UV-light even the whole rooms to sterilize Ebola containing surfaces. Gamma rays (1.2×10^6 rads) also inactivate Ebola virions present in liquid or solid materials; Gamma cells are available to irradiate materials. Boiling of materials for five minutes or exposing of materials to 60 degree Celsius for 30 min are also effective in inactivating Ebola. Diluted ethyl/methyl alcohol (10%), acetic acid (3%), chlorine solution (5%), Sodium hypochlorite (5.25%) and gluteraldehyde (1%) are readily available Ebola disinfectants. Ether, Sodium deoxycholate, β -propiolactones, 1,5-iodonaphthylazide, guanidium isothiocyanate also render Ebola virions in-infective (Burd 2015; Chiappelli *et al.*, 2015).

Measures to Counter Ebola Virus Disease

The reservoirs of Ebola virus variants, persistent in wild animal hosts, in areas of their endemicity, and acts of bioterrorism can be the cause of outbreaks of EVD in future. Secondary infections from Ebola infected foreign visitors/travellers could make such outbreaks pandemic. Counter measures against EVD

are urgently required to stem the ongoing outbreak in West Africa and any future outbreak(s) within or outside of the region of common existence of Ebolavirus. EVD would be best controlled by a single dose vaccination not requiring a booster dose and providing protection for as long as ten years. The other course is to counter EVD with antiviral therapeutics. Both vaccines and therapeutics are desired that can ward against Ebola infection and/or cure the EVD. Intensive efforts have been in progress in both the directions.

Anti-Ebola Vaccines

The general idea underlying the ongoing anti-Ebola vaccine development programme is to overexpress, in the vaccinated persons, Ebola proteins such as GP, VP40 and NP that are known to themselves cause no serious disease. GP is present on the surface of the virus and is highly immunogenic, but evolves faster than other genes (Jun *et al.*, 2015). The GP gene, GP and VP40 or GP and NP genes are recombinationally inserted into the genomes of viruses that do not cause serious disease or produce no or only minor side effects. GP, VP40 and NP proteins synthesized from Ebola vaccine virus genomes serve as small antigens to incite effective and B and T cell mediated anti-Ebola immunity (Becquart *et al.*, 2014; McElroy *et al.*, 2015). An Ebola virus whole genome (minus VP30) vaccine to prime host's immune system against many proteins of virus is also under testing. It is desired that the design of vaccines should be such that they can be produced readily in large quantities. There are several anti-Ebola vaccines under development. A few of these mentioned below have reached advanced clinical testing on human volunteers, following their effectivity tests on non human primates (Table 5).

VSV Δ G/EBOV GP or rVSV-ZEBOV vaccine: Presently, this is the most promising anti-Ebola vaccine. The Zaire Ebola GP gene is added on to the replication competent genome of Vesicular Stomatitis Virus (VSV, a rabies family zoonotic virus infective on insects, cattle, horse and pigs causing flu-like disease in humans) incapacitated for disease causation by deletion of its own glycoprotein gene. Non-human primates, both native and previously VSV vaccinated, were completely protected by intramuscular VSV Δ G/EBOV GP vaccination, against challenge of lethal dose of EBOV four weeks after vaccination. Post

vaccination, animals did not develop fever or any other adverse effect. It proved safe and effective on human volunteers and phase III trial is in progress (Marzi *et al.*, 2011, 2013, 2015a, b and c; Marzi and Feldmann 2014; Regules *et al.*, 2015). Recent final analysis of a ring vaccination in Guinea (West Africa) has indicated that rVSV-ZEBOV is effective and safe in preventing EVD at population level. Delivered via ring vaccination strategy, the vaccine is able to control EVD outbreak (Henao-Resrepo *et al.*, 2015 and 2016).

Ad-CAG opt ZGP and HPIV3/EboGP: These vaccines are another promising first line defence against EVD. In the Ad-CAG opt ZGP the vector is replication incompetent Adenovirus serotype 5 genome into which is inserted the ZEBOV GP gene optimized for over-expression in human cells. In HPIV3/EboGP, the vector for GP gene is human parainfluenza virus type 3. A single nasal spray of formulated either vaccine gave long lasting protection to non-human primates. The vaccine induced strong response in CD8+ and CD4+ T cells and Ebola GP specific antibodies in mucosa as well as systemically (Choi and Croyle 2013; Choi *et al.*, 2014; Meyer *et al.*, 2015). When available for human use, this type of non-injectable vaccines will have greater affordability due to simplification of transport, storage and administration of vaccine.

Ch-Ad3-EBOZ vaccine: Several versions of this vaccine are under development; they all have replication incompetent Chimpanzee Adenovirus type 3 genome as the vector, but vary in composition of the GP gene insert: GP of Zaire Ebola, GPs of Zaire and Sudan Ebola or GP of Marburg virus. These are used monovalently as well as bivalently (Zaire + Sudan and Marburg GP vaccines together). Intramuscular administration of a dose of Ch-Ad3-EBOZ followed by a booster dose of pox virus-GP vaccine eight weeks later gave full protection, as observed at 10 months after initial vaccination, in non-human primates (Kibuuka *et al.*, 2014; Ledgerwood *et al.*, 2014; O'Brien *et al.*, 2014; Stanley *et al.*, 2014; Sarwar *et al.*, 2015). Other booster vaccines to complement Ch-Ad3 based vaccines are under development (Rampling *et al.*, 2015).

VP30 minus whole genome vaccine: This vaccine uses whole Ebola virus from which VP30

gene has been deleted. The virus particles are inactivated with hydrogen peroxide. One dose of the vaccine protected cyanomolgous monkeys against the fatal dose of Ebola virus (Marzi *et al.*, 2015a and 2015b).

More than one kind of vaccine/s referred to here (and in Table 5) that are undergoing clinical trials in Africa are expected to be used and commercialized in the period of ongoing West Africa outbreak.

To control EVD outbreaks due to zoonotic transmission of Ebola virus to humans, development of animal species-specific anti-Ebola self-disseminating vaccine(s) is in progress. Animal species specific cytomegaloviruses, that have little or no effect on human health, are being altered to carry Ebola genes such as GP. Such vaccines are expected to immunize animals in the wild against Ebola virus infection (Murphy *et al.*, 2016; Marzi *et al.*, 2016).

Anti-Ebola Therapeutics

Presently, there is no specific treatment or drug for EVD that is proven to be safe and effective. Several types of treatments and medicines are under development; Tables 5 and 6 give a list of 138 chemicals/materials that are receiving attention. The thirty four putative cures of EVD included in Table 5 are those known to possess properties to antagonise the Ebola virions and/or expression products of Ebola virus genome, essential for the entry of virions into host cell and virus multiplication in host tissues and/or the virus infection induced host processes, leading to vascular damage and failure of multiple organs in host's body. Some of these are currently in different stages of clinical testing and several have been permitted by WHO for use on EVD patients on compassionate ground. The chemical structures of 16 of the putative anti-Ebola drugs mentioned in the Table 5 are shown in the Figs. 4 and 5. The table 6 lists 96 small molecules and γ -interferon, already in use as approved drugs for a variety of non-EVD human ailments, which have been identified as possible medicines for EVD, singly or in combination. There is experimental evidence that these drugs interfere with entry of Ebola into host cells and relieve some symptoms of EVD in model animals. From the list in Table 6, several compounds have entered into clinical trials for their use to cure EVD (www.who.int/medicines/emp_ebola_q/as/en/).

Among the six EVD-treatments (convalescent serum, DZNep, F4-6, Melatonin and Z Mapp and E4+E10 monoclonal antibodies) whose properties are summarized in Table 5, most promising appear to be Z Mapp alone, Z Mapp in combination with DZNep or interferon α and Z Mapp combined with convalescent serum; these require refrigeration and are administered through injections. Non-human primates that received Z Mapp five days after the lethal dose challenge of Ebola virus were found to be fully protected (Qiu *et al.*, 2014). These treatments have been prioritized for clinical trial. However, a subsequent study has raised doubts about the efficacy of Z Mapp. Kugelman *et al.*, (2015) observed that animals treated with MB-003, a Z Mapp-like antibody cocktail one or two days after Ebola infection, developed mutants of virus resistant to antibodies and succumbed to infection. However, single or combination of specific Ebola neutralizing antibodies isolated from the blood of EVD survivors are proving to be an effective treatment against infection by Bundibugyo, Zaire and Sudan Ebola virus (Burnholdt *et al.*, 2016; Corti *et al.*, 2016; Flyak *et al.*, 2016). Antibodies from filovirus patients collected 1-14 years after the primary disease have been observed to provide protections against filoviruses in general, including Ebola (Natesan, *et al.*, 2016). The antibody called mAb114 has been shown to completely protect Rhesus monkeys from lethal Zaire Ebola infection, when delivered intravenously for three consecutive days as late as five days after infection. Its protective action resulted from its ability to interact with receptor binding domain of the Ebola glycoprotein (Corti *et al.*, 2016; Misasi *et al.*, 2016).

Another group of compounds that hold promise are the drugs of choice against heterologous viral diseases, which demonstrate pronounced anti-Ebola activity. Antivirals BCX4430, GS-5734, Brincidofovir and Favipiravir (Table 5) are also prioritized candidates for clinical trials against EVD; these are stable at room temperature and can be given orally. Another class of drugs receiving impetus for further testing are TKM-Ebola types and AVI-7537, RNAs antiparallel to specific Ebola essential gene mRNAs leading to loss of function of the latter and arrest of Ebola multiplication (Table 5). Blocking of human calcium signalling pathway, dependent on *STIM1* and *ORAI1* genes, that is essential for virus escape/budding from host cells, by compounds such as 5D

(5, N-[2, 2, 2-trichloro-1-(2-naphthylamino)ethyl]-2 formamide) has been proposed as a potent therapeutic for EVD. Search for potent ORA1 is in progress (Han *et al.*, 2015).

Re-purposing tests on thousands of drugs have revealed that compounds with diverse mode of action on human body (Table 6), variously prescribed for treatment of infectious diseases, disorders of different organs of body, cancers and depression, possess anti-Ebola activity (Litterman *et al.*, 2015). They offer possibilities of combinatorial usage to harvest their synergism against Ebola. In the first instance, Azithromycin (antibacterial), Sunitinib (tyrosine kinase inhibitor), Amiodarone (Na^+ channel blocker), Clomiphene (estrogen receptor modulator), Chloroquine (antimalarial), Bepridil (Calcium channel blocker) and Sertraline (serotonin uptake inhibitor) are being taken up/ recommended for clinical testing against EVD (www.who.int/medicines/emp_ebola_qas/en/; Johansen *et al.*, 2015). Low doses of Imatinib, a drug related to Sunitinib, stimulate the bone marrow to produce more of neutrophils and macrophages to resist infections (Napier *et al.*, 2015). Patients given the generic statin drug atorvastatin (40 mg/day) and the angiotensin receptor blocker irbesartan (150 mg/day) survived EVD 100% (Opal *et al.*, 2015). The treatment restored the endothelial barrier integrity.

The complementary approaches of designing and re-purposing are bound to yield drug(s) suitable for pre- and post-infection, respectively, to protect against Ebola infection and treat EVD. The experimental observations on re-purposing of medicines summarized in Table 6 and corresponding results of re-purposing of approved and experimental drugs show that a minimum of 43 (perhaps hundreds) of host genome coded functions interact with one or more of nine proteins coded by Ebola genome (Cheng *et al.*, 2015; Veljkovic *et al.*, 2015). Study of Ebola infection in host cells or whole organisms in the presence of Ebola virus inhibiting compounds is expected to reveal the roles that various host genes play in Ebola virus biology.

Life Saving Supportive Care

Until vaccine and medicine based therapies for EVD become available, supportive medical care, which nurses patients to limit intensity of disease in his/her body, remains the main treatment. The role of

experimental therapies (such as Z Mapp, BCX4430, Favipiravir, Brincidofovir and F4-6 etc) is not yet firmly established, although their compassionate use has been in vogue. Fig. 6 gives a profile of the currently available, experience based, supportive care treatments of EVD. Supportive treatment essentially consists of ensuring that patient's body maintains fluid volume, balance of electrolytes, oxygen status, blood pressure and kidney function (Hunt *et al.*, 2015). It has been noted that the EVD patients who start to receive supportive care as soon as the post-incubation period, early symptoms appear have better chances of survival than those who receive the medicinal care in late stages of infection. Recently, Chiappelli *et al.*, (2015) have advised inclusion of Selenium replishment as an element of palliative care. Patients under supportive care take months to recover. Only those are considered as cured in whom virus is found absent from their blood/plasma and other body fluids. The so-called cured/convalescent patients require continued attention for years about one or more of the following medical ailments: psychosis, photophobia, excessive tearing from eyes, sloughing of skin, hairloss, deafness, myelitis, pericarditis, orchitis, hepatitis and secondary viral, bacterial, fungal or other infections.

Measures to Adopt for Containment of Ongoing Outbreak(S) and Prevention of New Outbreaks of Ebola Virus Disease (EVD)

In the absence of prophylactic vaccines and medicines, EVD is extremely dangerous to human populations on account of the Ebola virus being highly stable and infectious and especially virulent on humans. Chain of person-to-person infection, starting from one Ebola infected human is known to cause EVD outbreaks of large magnitude, such as the present EVD outbreak in West Africa. This outbreak first noted in Guinea in February 2014, has by 31 March 2016, infected over 28639 persons, out of which 11316 have died in Guinea and neighbouring countries Sierra Leone and Liberia. From these countries, EVD got imported into the African countries (Mali, Senegal and Nigeria) and into several countries of Europe (Spain, Italy, France, Britain, Germany, Switzerland, Netherland and Norway) and into Canada and USA. Here (www.msf.org/article/ebola-) are given definition of EVD patients, procedure for identification and quarantining of EVD patients, barrier nursing of EVD

patients, disposal of contaminated materials and cadavers and precautions against any deliberate or accidental EVD attack. The concepts are summarized in the Fig. 7.

EVD Patient

The Ebola virus infection spreads from symptomatic EVD patients. Ebola virus infected persons (patients) develop EVD symptoms after an incubation period of 21/23 days or less. Within one week of the onset of initial EVD symptoms, which are mild and simulate those of several other ailments/infective diseases, the viremia peaks and EVD patient becomes highly infective. Direct contact with such a EVD patient's body or any of his/her secretions/excretions, including the matter liberated into air while coughing and sneezing, can cause infection. Viremic EVD patients who are not bed-ridden spread infection by coming in contact with non-infected in crowded places and to relatives, friends and work colleagues. Upon worsening of EVD symptoms, patient's hospitalization exposes healthcare workers and visitors to hospital to EVD infection transmission. Practice of conventional rituals at the time of funeral of dead EVD transmission is another occasion for extensive EVD transmission (Ebola-RRA-WestAfrica-8April2014.pdf). As a rule of thumb, a person who comes within one metre of living symptomatic EVD patient or cadaver of a person who died of EVD is a suspected case of Ebola infection or EVD patient.

Secure Quarantining of EVD Patients at Health Care Centres/hospitals

EVD can be contained by strictly controlling secondary transmission by identifying and isolating the suspected EVD patients in secure quarantine facilities (Fig. 7) (Roddy *et al.*, 2012; Chowell *et al.*, 2015). All persons in the geographical region of outbreak who show initial symptoms of EVD and are diagnosed EVD positive will be isolated and treated in specially created wards in identified hospitals. Any traveller who have been to the geographical region of a EVD outbreak over the last three to four weeks period and shows higher than normal body temperature ($\geq 37.5^{\circ}\text{C}$) will be called a suspected EVD patient and quarantined (European Food Safety Authority 2014; European Centre for Disease Prevention and Control 2014). Other travellers from the outbreak areas and those who came in contact with travellers

from outbreak areas will be observed for development of symptoms over next three to four weeks. Those that develops symptoms will be quarantined, diagnostically tested and confirmed EVD patients will be isolated and treated. The contacts of such patients will be tracked, observed twice daily for body temperature and attended to as per the above EVD patient criterion, *ad infinitum* (www.virology.ws/2014/10/16/the-quarantine-period-for-ebola-virus/).

People in the areas of outbreak and in general need to be familiarized with the disease and mechanisms of its transmission. Suspected patients should refrain from breast-feeding and unprotected sex and offer themselves for isolation and diagnosis. Homes of patients after they are quarantined or admitted in hospital should be disinfected.

Barrier Nursing of EVD Patients

Doctors, nurses and other health care givers, including family members/ friends of patients to be engaged for providing medical care to EVD patients must get extensively familiarized about EVD, such that chances of their getting infected are minimised. Towards this end, they must also be provided with pre-prepared protocols and some training. The health care staff, before entering the quarantine or patient care area and while providing medical attention to EVD patients, must wear personal protective equipment (PPE), that leaves no part of skin unprotected. The PPE to be worn includes single use water/fluid-resistant hooded gown/coverall, boot covers that extend upto mid-calf and apron, two sets of nitrile gloves with extended cuffs, full face shield, helmet covered with disposable hood fitted with N95 respirator. When performing any aerosol-generating procedure, a powered air purifier respirator (PAPR) suit may be worn in place of N95 respirator. Training in donning, doffing and use of PPE without accidents is essential (Reiter *et al.*, 1999; Funk and Kumar 2015 and www.phac-aspc.gc.ca/lab-bio/res/psds-ffss/ebola-eng.php).

Disposal of Materials Used Upon or by EVD Patients and Their Sewage and Cremation of Diseased Corpses

Best procedure to respectively dispose of or decontaminate the medical waste and clothing, eating utensils, linen and other belonging of EVD patients is by incineration or autoclaving in suitably designed

devices of large size. Sewage of the quarantined EVD patients or of those hospitalized in special wards should be collected and stored for atleast two weeks at 25°C and one week at 30°C for decay of Ebolavirus to occur to an extent of $\geq 99.99\%$ (Bibby *et al.*, 2015; Casanova and Weaver 2015; Judson *et al.*, 2015). The bodies of the dead EVD patients should be handled by PPE wearing persons. Cremation in closed container would be the best means of containing spread of infection that can result from the conventional funeral.

Steps to Avoid Impending Ebolavirus Disease Epidemic in India

On account of their stability, rapid transmissibility and fatal disease causing properties, Ebolaviruses have been described as class A bioterror agents. Ebolaviruses are reservoired among the forest animals in Africa. Since it is impossible to eliminate the Ebola reservoir in wild forest animals, EVD outbreaks are expected to occur periodically in different countries of Africa. Eating semi-cooked Ebolavirus containing bush meat which is traded (Maughan 2014) is the main and continuing factor for Ebolavirus outbreaks in Africa (European Centre for Disease Prevention and Control 2014; Casanova and Weaver 2015). Importation of EVD into countries outside of areas of outbreak will remain a lurking danger. Entry and spread of Ebolavirus in densely populated cities of India will be disastrous. Residents of super-densely-populated shanty/slum colonies in the cities will be especially vulnerable to Ebolavirus infection. Within India, virus infection could spread from its epicentre to other cities by movement of infected persons. The general absence of immunity against Ebolavirus and population dynamics in densely populated areas in India dictate that the country should prepare for any sudden challenge of EVD importation (Kumar and Gopal 2014).

A variety of steps, listed below, should be taken urgently and the process improved on a continous basis. Guidelines prepared by the World Health Organization, Centre for Disease Control (CDC, USA) and National Institute of Allergy and Infectious Diseases (NIAID, USA) (<http://touch.govexec.com/govexec/#1/entry/ebola-discussion-moves-how-to-prevent-the-next-epideme>, 550ae.; WHO/EVD/Guidance/Sur Non E Count/14.1) are required to be strictly implemented

for the safety of travellers and surveillance of travellers by immigration officials and ship and airline services (Jacobson *et al.*, 2016). General public needs to be made aware of the EVD and hygienic control of infection. The topic of infectious diseases, including EVD should be now included in the curricula of school education. Medical and nursing colleges must have practical and theory courses emphasizing on the emerging infectious diseases, including Ebola. Teams of doctors, nurses and epidemiologists must be composed in all the states and union territories and suitably trained about management of EVD (Funk and Kumar 2015). Diagnostic laboratories should be established in all the major cities. Mobile diagnostic laboratories should also be constructed with desired safety levels for conducting tests, at foci of disease spread. Hospitals should be identified which will function as the EVD treatment centres under whose charge quarantine facilities will become operational on short notice. Procedures for fool proof case management, contact tracing, quarantining, treatment and safe disposal of contaminated materials and cadavers will be standardized, published and kept ready. The materials to be used in treatment, safeguarding of care givers and prophylaxis will be stockpiled and steps taken to inventorise their speedy procurement and/or manufacture. Some Indian laboratories should initiate research on development of pre- and post-infection drugs effective in preventing and treating EVD, using suitable containment facilities (P4). This work should be broad based against communicable diseases, since there is evidence that some of the therapies may be common to several different virus caused diseases (Table 5).

Quarantining of infected people is considered the most effective mode for stopping the spread of Ebola virus disease outbreak. To arrest the spread of disease, areas housing the infected persons will need to be isolated from the rest. The needs of quarantined people will be met as above. The procedure to combat EVD epidemic will have to be updated as the vaccines and therapies already at the advanced stages of clinical trial get approved and begin to get manufactured.

Summary and Concluding Remarks

The four known species of filoviridae viral genus Ebola occur in the form of enveloped tubular virion in which 19 kb negative sense single strand RNA genome is

encapsulated. Ebola virus genome has seven cistrons that express nine functional proteins/peptides. Ebola viruses are zoonotic. Ebola species, such as Zaire Ebola virus (ZEBOV), which cause highly lethal disease (called Ebola virus disease, EVD) are presently reservoirized in wild animals of There have been more than 20 recorded outbreaks of EVD in Africa in last four decades. Till the Ebola reservoir exists, EVD outbreaks will continue to occur sporadically. An outbreak initiates when a human gets exposed to blood or body fluids of infected animal(s) (bat, primate etc.). It then spreads person-to-person nosocomially.

Ebola virions attach to and enter into the cytoplasm of mononuclear phagocytic cells present at the site of infection in mucosal tissue or injured skin, opportunistically. The virus expresses its genes, replicates and assembles pro-virions in host cell cytoplasm which mature at and get released as infective particles from host cell's outer plasma membrane. As the virus multiplies in the dendritic and macrophage cells, the interferons based innate antiviral immune response pathway is overcome by Ebola protein functions. Failure of dyeing infected dendritic cells to activate the adaptive immune response system and consequent T-cell apoptosis and disabling of - cells and neutrophils allows the virus to multiply rapidly. Immunity suppression allows virus to spread into various organs of the host through blood stream and lymphatic channels. Host launches a pro-inflammatory response by release of chemokines and cytokines. This results in gastro-intestinal dysfunction, coagulation defects, vascular leakages, necrotic failure of organs, drop in blood pressure and shock, altogether leading to death of EVD patient. Presently, there is no approved vaccine or specific medicine for EVD. However, several vaccines, anti-viral approaches and medicines effective in preventing and/or curing EVD are under various stages of clinical trial. Patients, who are detected with EVD early and who receive symptomatic supportive care often survive. Ebola species have been observed to be accumulating mutations in their genome for increased persistence in humans, like in their reservoir hosts.

Ebola virus is a biological weapon because its particles are stable *in vitro* and EVD has long incubation period; one infected person, if not checked, can be responsible for EVD epidemic. Densely

populated localities/countries are especially vulnerable for rapid spread of EVD. World Health Organization and health agencies of several countries have developed guidelines/protocols for stopping ongoing EVD outbreaks and their importation to countries of continents other than Africa. India-suited guidelines need to be developed and their strict pursuance can avoid invasion of Ebola into India and stop any outbreak if it occurs. To meet any future EVD challenge, India should manufacture/stockpile PPE and supportive care medicines. Emerging infectious diseases, including Ebola, should be included in the curricula of school and college education and general public should be made aware of aspects of EVD. Infectious disease laboratories should initiate work on vaccines and

medicines for EVD. Preparedness can ward-off impending disaster to economy and development of countries and health of their people that may be caused by importation of EVD epidemic.

Acknowledgements

Grateful thanks are due towards Indian National Science Academy for the grant of an INSA emeritus scientistship to SK and to Director of National Institute of Plant Genome Research and Director of Indian Institute of Chemical Technology for facilities, and to the virologists AC Banerjee of National Institute of Immunology and RK Saxena of South-Asian University for reading the draft of the manuscript and making useful suggestions for its improvement.

References

- Acha P N and Szyfres B (2003) In Pan American Health Organization (Ed.), *Zoonoses and Communicable Diseases Common to Man and Animals* (3rd ed., pp. 142-145). Washington D.C.: Pan American Health Organization
- Adu-Gyamfi E, Soni S P, Xue Y, Digman M A, Gratton E and Stahelin R V (2013) The Ebola virus matrix protein penetrates into the plasma membrane: a key step in viral protein 40 (VP40) oligomerization and viral egress *J Biol Chem* **288** 5779-5789
- Adu-Gyamfi E, Soni S P, Jee C S, Digman M A, Gratton E and Stahelin R V (2014) A loop region in the N-terminal domain of Ebola virus VP40 is important in viral assembly, budding, and egress *Viruses* **6** 3837-3854
- Agnandji S T *et al.* (2016) Phase I trials of rVSV Ebola vaccine in Africa and Europe *New Engl J Med* **374** 1647-1660
- Ahrberg C D, Manz A and Newzil P (2016) Palm-sized device for point-of-care Ebola detection *Analyt Chem* **88** 4803-4807
- Alfson K J, Worwa G, Carrion R Jr and Griffiths A (2016) Determination of the spontaneous mutation frequency of Ebola virus and exploitation of this therapeutically *J Virol* 02701-15
- Allela L, Bourry O, Pouillot R, Delicat A, Yaba P, Kumulungui B, Rouquet P, Gonzalez J P and Leroy E M (2005) Ebola virus antibody prevalence in dogs and human risk *Emerg Infect Dis* **11** 385-390
- Alvarez C P, Lasala F, Carrillo J, Muniz O, Corbi A L and Delgado R (2014) C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans *J Virol* **76** 6841-6844
- Ansari AA (2014) Clinical features and pathobiology of Ebolavirus infection *J Autoimmun* **55** 1-9
- Ascenzi P, Bocedi A, Heptonstall J, Capobianchi M R, Di Caro A, Mastrangelo E, Bolognesi M and Ippolito G (2008) Ebolavirus and Marburgvirus: insight the Filoviridae family *Mol Aspects Med* **29** 151-185
- Audet J and Kobinger G P (2015) Immune evasion in ebolavirus infections *Viral Immunol* **28** 10-18
- Baize S, Leroy E M, Georges A J, Georges-Courbot M C, Capron M, Bedjabaga I, Lansoud-Soukate J and Mavoungou E (2002) Inflammatory responses in Ebola virus-infected patients *Clin Exp Immunol* **128** 163-168
- Baize S, Leroy E M, Mavoungou E and Fisher-Hoch S P (2000) Apoptosis in fatal Ebola infection. Does the virus toll the bell for immune system? *Apoptosis* **5** 5-7
- Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, Soropogui B *et al.* (2014) Emergence of Zaire Ebola virus disease in Guinea *N Engl J Med* **371** 1418-1425
- Barrette R W, Metwally S A, Rowland J M, Xu L, Zaki S R, Nichol S T, Rollin P E, Towner J S *et al.* (2009) Discovery of swine as a host for the Reston ebolavirus *Science* **325** 204-206
- Barton C, Kouokam J C, Lasnik A B, Foreman O, Cambon A, Brock G, Montefiori D C, Vojdani F, McCormick A A, O'Keefe B R and Palmer K E (2014) Activity of and effect of subcutaneous treatment with the broad-spectrum antiviral lectin griffithsin in two laboratory rodent models. *Antimicrob Agents Chemother* **58** 120-127

- Basler C F, Wang X, Muhlberger E, Volchkov V, Paragas J, Klenk H D, García-Sastre A and Palese P (2000a) The Ebola virus VP35 protein functions as a type I IFN antagonist *Proc Natl Acad Sci USA* **97** 12289-12294
- Basler C F, Mikulasova A, Martinez-Sobrido L, Paragas J, Muhlberger E, Bray M, Klenk H D, Palese P and Garcia-Sastre A (2003) The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3 *J Virol* **77** 7945-7956
- Basler C F and Palese P (2004) Modulation of innate immunity by filoviruses. In: Klenk H D, Feldmann H, editors. Marburg and Ebola viruses *Norfolk: Horizon Bioscience* 305-349
- Basler C F, Wang X, Muhlberger E, Volchkov V, Paragas J, Klenk H D, Garcia-Sastre A and Palese P (2000b) The Ebola virus VP35 protein functions as a type I IFN antagonist *Proc Natl Acad Sci USA* **97** 12289-12294
- Baum A and Garcia-Sastre A (2011) Differential recognition of viral RNA by RIG-I *Virulence* **2** 166-169
- Bausch D G and Schwarz L (2014) Outbreak of ebola virus disease in Guinea: where ecology meets economy *PLoS Negl Trop Dis* **8** e3056
- Bausch D G, Towner J S, Dowell S F, Kaducu F, Lukwiya M, Sanchez A, Nichol S T, Ksiazek T G and Rollin P E (2007) Assessment of the risk of Ebola virus transmission from bodily fluids and fomites *J Infect Dis* **196** Suppl 2 S142-147
- Becquart P, Mahlakoiv T, Nkoghe D and Leroy E M (2014) Identification of continuous human B-cell epitopes in the VP35, VP40, nucleoprotein and glycoprotein of Ebola virus *PLoS One* **9** e96360
- Bellan S E, Pulliam J R, Dushoff J and Meyers L A (2014) Ebola control: effect of asymptomatic infection and acquired immunity *Lancet* **384** 1499-1500
- Belyi V A, Levine A J and Skalka A M (2010) Unexpected inheritance: multiple integrations of ancient bornavirus and ebolavirus/marburgvirus sequences in vertebrate genomes *PLoS Pathog* **6** e1001030
- Bhakuni D S, Jain S and Singh A N (1980) Biosynthesis of the bisbenzylisoquinoline alkaloid, tetrandrine *Phytochemistry* **19** 2347-2350
- Bharat T A, Noda T, Riches J D, Kraehling V, Kolesnikova L, Becker S, Kawaoka Y and Briggs J A (2012) Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography *Proc Natl Acad Sci USA* **109** 4275-4280
- Bhattacharyya S, Mulherkar N and Chandran K (2012) Endocytic pathways involved in filovirus entry: advances, implications and future directions *Viruses* **4** 3647-3664
- Biava M, Caglioti C, Bordi L, Castilletti C, Colavite F, Quartu S *et al.* (2017). Detection of viral RNA in tissues following plasma clearance from an Ebola virus infected *PLoS Pathol.*, **13** e1006065.
- Bibby K, Casson L W, Stachler E, Charles N and Haas C N (2015) Ebola virus persistence in the environment: state of the knowledge and research needs *Environ Sci Technol Lett* **2** 2-6
- Biedenkopf N, Hartlieb B, Hoenen T and Becker S (2013) Phosphorylation of Ebola virus VP30 influences the composition of the viral nucleocapsid complex: impact on viral transcription and replication *J Biol Chem* **288** 11165-11174
- Blaney J E, Marzi A, Willet M, Papaneri A B, Wirblich C, Feldmann F, Holbrook M, Jahrling P, Feldmann H and Schnell M J (2013) Antibody quality and protection from lethal Ebola virus challenge in nonhuman primates immunized with rabies virus based bivalent vaccine *PLoS Pathog* **9** e1003389
- Bornholdt Z A, Turner H L, Murin C D, Li W, Sok D, Souders C A *et al.* (2016) Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak *Science* **351** 1078-1083
- Booth J L, Roberts D R, Tavallai C S, Jean S, Fidanza A, Cruz-Luna T, Siembiba P, Cycon K A, Cornelissen C N, Dent P (2015) GRP78/BiP/HSPA5/Dna K is a universal therapeutic target for human disease *J Cell Physiol* **230** 1661-1676
- Botcher J P, Beyer M, Meissner F, Abdullah Z, Sander J, Hochst B *et al.* (2015) Functional classification of memory CD8(+) T cells by CX3CR1 expression *Nat Commun* **6** 8306
- Bradfute S B, Swanson P E, Smith M A, Watanabe E, McDunn J E, Hotchkiss R S and Bavari S (2010) Mechanisms and consequences of ebolavirus-induced lymphocyte apoptosis *J Immunol* **184** 327-335
- Briese T, Kapoor A, Mishra N, Jain K, Kumar A, Jabado O J and Lipkin W I (2015) Virome capture sequencing enables sensitive viral diagnosis and comprehensive virome analysis *MBio*. **6** pii: e01491-15
- Bray M, Raymond J L, Geisbert T and Baker R O (2002) 3-deazaneplanocin A induces massively increased interferon-alpha production in Ebola virus-infected mice *Antiviral Res* **55** 151-159
- Broadhurst M J, Kelly J D, Miller A, Semper A, Bailey D, Gropelli E *et al.* (2015) ReEBOV Antigen Rapid Test kit for point-of-care and laboratory-based testing for Ebola virus disease: a field validation study *Lancet* **386** 867-874
- Burd E M (2015) Ebola virus: a clear and present danger *J Clin*

- Microbiol* **53** 4-8
- Cai H, Parks J W, Wall T A, Stott M A, Stambaugh A, Alfson K *et al.* (2015) Optofluidic analysis system for amplification-free, direct detection of Ebola infection *Sci Rep* **5** 14494
- Cenedella R J (2009) Cholesterol synthesis inhibitor U18666A and the role of sterol metabolism and trafficking in numerous pathophysiological processes *Lipids* **44** 477-487
- Cardenas W B, Loo Y M, Gale M Jr, Hartman A L, Kimberlin C R, Martínez-Sobrido L, Saphire E O and Basler C F (2006) Ebola virus VP35 protein binds double-stranded RNA and inhibits alpha/beta interferon production induced by RIG-I signaling *J Virol* **80** 5168-5178
- Cardona-Maya W D, Hernandez P A V and Henao D E (2014) Male Ebola Survivors: Do not forget to use a condom *Reproductive Sci* DOI: 10.1177/1933719114563733
- Carette J E, Raaben M, Wong A C, Herbert A S, Obernosterer G, Mulherkar N *et al.* (2011) Ebola virus entry requires the cholesterol transporter Niemann-Pick C1 *Nature* **477** 340-343
- Carroll S A, Towner J S, Sealy T K, McMullan L K, Khristova M L, Burt F J, Swanepoel R, Rollin P E and Nichol S T (2013) Molecular evolution of viruses of the family Filoviridae based on 97 whole-genome sequences *J Virol* **87** 2608-2616
- Casanova L M and Weaver S R (2015) Inactivation of an enveloped surrogate virus in human sewage *Environ Sci Technol Lett* **2** 76-78
- Chan S Y, Empig C J, Welte F J, Speck R F, Schmaljohn A, Kreisberg J F and Goldsmith M A (2001) Folate receptor-alpha is a cofactor for cellular entry by Marburg and Ebola viruses *Cell* **106** 117-126
- Chandran K, Sullivan N J, Felbor U, Whelan S P and Cunningham J M (2005) Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection *Science* **308** 1643-1645
- Chang J, Guo J T, Du Y and Block T (2013a) Imino sugar glucosidase inhibitors as broadly active anti-filovirus agents *Emerg Microbes Infect* **2** e77
- Chang J, Warren T K, Zhao X, Gill T, Guo F, Wang L, Comunale M A *et al.* (2013b) Small molecule inhibitors of ER α -glucosidases are active against multiple hemorrhagic fever viruses *Antiviral Res* **98** 432-440
- Chang T H, Kubota T, Matsuoka M, Jones S, Bradfute S B and Bray M, Ozato K (2009) Ebola Zaire virus blocks type I interferon production by exploiting the host SUMO modification machinery *PLoS Pathog* **5** e1000493
- Cheng H, Lear-Rooney C M, Johansen L, Varhegyi E, Chen Z W, Olinger G G and Rong L (2015) Inhibition of Ebola and Marburg virus entry by G protein-coupled receptor antagonists *J Virol* **89** 9932-9938
- Chiappelli F, Bakhordarian A, Thames A D, Du A M, Jan A L, Nahcivan M *et al.* (2015) Ebola: translational science considerations *J Transl Med* **13** 11
- Chen G, Koellhoffer J F, Zak S E, Frei J C, Liu N, Long H, Ye W, Nagar K, Pan G, Chandran K, Dye J M, Sidhu S S and Lai J R (2014) Synthetic antibodies with a human framework that protect mice from lethal Sudan ebolavirus challenge *ACS Chem Biol* **9** 2263-2273
- Cheng A C and Kelly H (2014) Are we prepared for Ebola and other viral haemorrhagic fevers? *Aust N Z J Public Health* **38** 403-404
- Choi J H and Croyle M A (2013) Emerging targets and novel approaches to Ebola virus prophylaxis and treatment *Bio Drugs* **27** 565-583
- Choi J H, Jonsson-Schmunk K, Qiu X, Shedlock D J, Strong J, Xu J X, Michie K L, Audet J *et al.* (2014) A single dose respiratory recombinant adenovirus-based vaccine provides long-term protection for non-human primates from lethal Ebola infection *Mol Pharm* DOI: 10.1021/mp500646d
- Chowell D, Castillo-Chavez C, Krishna S, Qiu X and Anderson K S (2015) Modelling the effect of early detection of Ebola *The Lancet Infectious Diseases* **15** 148-149
- Clark D V, Jahrling P B and Lawler J V (2012) Clinical management of Filovirus-infected patients *Viruses* **4** 1668-1686
- Cohen E (2014) Doctor treats Ebola with HIV drug in Liberia—seemingly successfully. edition.cnn.com/2014/09/27/health
- Corti D, Misasi J, Mulangu S, Stanley D A, Kanekiyo M, Wollen S, Ploquin A *et al.* (2016) Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody *Science* **351** 1339-1342
- Cote M, Misasi J, Ren T, Bruchez A, Lee K, Filone C M, Hensley L, Li Q, Ory D, Chandran K and Cunningham J (2011) Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection *Nature* **477** 344-348
- Crary S M, Towner J S, Honig J E, Shoemaker T R and Nichol S T (2003) Analysis of the role of predicted RNA secondary structures in Ebola virus replication *Virology* **306** 210-218
- Currie J, Grenfell B and Farrar J (2016) Infectious diseases. Beyond Ebola *Science* **351** 815-816
- Daugherty M D and Malik H S (2014) How a virus blocks a cellular emergency access lane to the nucleus, STAT *Cell Host Microbe* **16** 150-152

- de La Vega M A, Wong G, Kobinger G P and Qiu X (2015) The multiple roles of sGP in Ebola pathogenesis *Viral Immunol* **28** 3-9
- Diehl W E, Lin A E, Gurubagh N D, Carvalho L M, Kim K, Kyawe P P *et al.* (2016) Ebola virus glycoprotein with increased infectivity dominated the 2013-2016 epidemic. *Cell* **167** 1088-1098
- Dolnik O, Kolesnikova L and Becker S (2008) Filoviruses: Interactions with the host cell *Cell Mol Life Sci* **65** 756-776
- Dolnik O, Volchkova V, Garten W, Carbonnelle C, Becker S, Kahnt J, Stroher U, Klenk H D and Volchkov V (2004) Ectodomain shedding of the glycoprotein GP of Ebola virus *EMBO J* **23** 2175-2184
- Dong S, Yang P, Li G, Liu B, Wang W, Liu X, Xia B, Yang C, Lou Z, Guo Y and Rao Z (2015) Insight into the Ebola virus nucleocapsid assembly mechanism: crystal structure of Ebola virus nucleoprotein core domain at 1.8 Å resolution *Protein and Cell* **6** 351
- Dowall S D, Matthews D A, Garcia-Dorival I, Taylor I, Kenny J, Hertz-Fowler C, Hall N, Corbin-Lickfett K *et al.* (2014) Elucidating variations in the nucleotide sequence of Ebola virus associated with increasing pathogenicity *Genome Biol* **15** 540
- Duan D, Fan K, Zhang D, Tan S, Liang M, Liu Y, Zhang J *et al.*, (2015) Nanozyme-strip for rapid local diagnosis of Ebola *Biosens Bioelectron* **74** 134-141
- Dziubanska P J, Derewenda U, Ellena J F, Engel D A and Derewenda Z S (2014) The structure of the C-terminal domain of the Zaire ebolavirus nucleoprotein *Acta Crystallogr D Biol Crystallogr* **70** 2420-2429
- Ebihara H, Zivcec M, Gardner D, Falzarano D, LaCasse R, Rosenke R, Long D, Haddock E, Fischer E, Kawaoka Y and Feldmann H (2013) A Syrian golden hamster model recapitulating ebola hemorrhagic fever *J Infect Dis* **207** 306-318
- Escudero-Perez B, Volchkova V A, Dolnik O, Lawrence P and Volchkov V E (2014) Shed GP of Ebola virus triggers immune activation and increased vascular permeability *PLoS Pathog* **10** e1004509
- European Food Safety Authority (2014) An update on the risk of transmission of Ebola virus (EBOV) via the food chain *EFSA J* **12** 3884
- European Centre for Disease Prevention and Control (2014) Infection prevention and control measures for Ebola virus disease: Entry and exit body temperature screening measures: ECDC Stockholm
- Fabozzi G, Nabel C S, Dolan M A and Sullivan N J (2011) Ebolavirus proteins suppress the effects of small interfering RNA by direct interaction with the mammalian RNA interference pathway *J Virol* **85** 2512-2523
- Falzarano D, Krokhin O, Wahl-Jensen V, Seebach J, Wolf K, Schnittler H J and Feldmann H (2006) Structure-function analysis of the soluble glycoprotein, sGP, of Ebola virus *Chembiochem* **7** 1605-1611
- Fauci A S (2014) Ebola - underscoring the global disparities in health care resources *N Engl J Med* **371** 1084-1086
- Faye O, Faye O, Soropogui B, Patel P, El Wahed A A, Loucoubar C, Fall G, Kiory D *et al.* (2015) Development and deployment of a rapid recombinase polymerase amplification Ebola virus detection assay in Guinea in 2015 *Euro Surveill* **20** doi: 10.2807/1560-7917.ES.2015.20.44.30053
- Fedson D S (2013) Treating influenza with statins and other immunomodulatory agents *Antiviral Res* **99** 417-435
- Fedson D S (2015) A practical treatment for patients with Ebola virus disease *J Infect Dis* **211** 661-662
- Fedson D S, Jacobson J R, Rordam O M and Opal S M (2015) Treating the host response to Ebola virus disease with generic statins and angiotensin receptor blockers *MBio* **6** e00716
- Feldmann H and Geisbert T W (2011) Ebola haemorrhagic fever *Lancet* **377** 849-862
- Feldmann H, Geisbert T and Kawaoka Y (2007) Filoviruses: recent advances and future challenges *J Infect Dis* **196** (suppl 2) S129-S443
- Feldmann H, Volchkov V E, Volchkova V A, Stroher U and Klenk H D (2001) Biosynthesis and role of filoviral glycoproteins *J Gen Virol* **82** 2839-2848
- Feng Z, Cerveny M, Yan Z and He B (2007) The VP35 protein of Ebola virus inhibits the antiviral effect mediated by double-stranded RNA-dependent protein kinase PKR *J Virol* **81** 182-192
- Fisher-Hoch S P, Platt G S, Neild G H, Southee T, Baskerville A, Raymond R T, Lloyd G and Simpson D I (1985) Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola) *J Infect Dis* **152** 887-894
- Fletcher T E, Fowler R A and Beeching N J (2014) Understanding organ dysfunction in Ebola virus disease *Intensive Care Med* **40** 1936-1939
- Florescu D F and Keck M A (2014) Development of CMX001 (Brincidofovir) for the treatment of serious diseases or conditions caused by dsDNA viruses *Expert Rev Anti Infect Ther* **12** 1171-1178

- Flyak A I, Shen X, Murin C D, Turner H L, David J A, Fusco M L, Lampley R *et al.* (2016) Cross-reactive and potent neutralizing antibody responses in human survivors of natural Ebola virus infection *Cell* **164** 392-405
- Fowler R A, Fletcher T, Fischer W A, Lamontagne F, Jacob S, Brett-Major D *et al.* (2014) Caring for critically ill patients with ebola virus disease. Perspectives from West Africa *Am J Respir Crit Care Med* **190** 733-737
- Francica J R, Varela-Rohena A, Medvec A, Plesa G, Riley J L *et al.* (2010) Steric shielding of surface epitopes and impaired immune recognition induced by the Ebola virus glycoprotein *PLoS Pathog* **6** e1001098
- Funk D J and Kumar A (2015) Ebola virus disease: an update for anesthesiologists and intensivists *Can J Anaesth* **62** 80-91
- Gallaher W R and Garry R F (2015) Modeling of the ebola virus delta Peptide reveals a potential lytic sequence motif *Viruses* **7** 285-305
- Gao X, Rong G, Lu Y, Duan X and Duan H (2016) Symptoms closely associated with Ebola virus disease *Lancet Infect Dis* **16** 18-19
- Garcia M, Cooper A, Shi W, Bornmann W, Carrion R, Kalman D and Nabel G J (2012) Productive replication of Ebola virus is regulated by the c-Abl1 tyrosine kinase *Sci Transl Med* **4** 23ra24
- Garcia-Dorival I, Wu W, Dowall S, Armstrong S, Touzelet O, Wastling J, Barr J N, Matthews D, Carroll M, Hewson R and Hiscox J A (2014) Elucidation of the Ebola Virus VP24 cellular interactome and disruption of virus biology through targeted inhibition of host-cell protein function *J Proteome Res* **13** 5120-5135
- Gehring G, Rohrmann K, Atenchong N, Mittler E, Becker S, Dahlmann F, Pohlmann S *et al.* (2014) The clinically approved drugs amiodarone, dronedarone and verapamil inhibit filovirus cell entry *J Antimicrob Chemother* **69** 2123-2131
- Geisbert T W, Hensley L E, Larsen T, Young H A, Reed D S, Geisbert J B *et al.* (2003a) Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection *Am J Pathol* **163** 2347-2370
- Geisbert T W, Hensley L E, Gibb T R, Steele K E, Jaax N K and Jahrling P B (2000) Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses *Lab Invest* **80** 171-186
- Geisbert T W, Hensley L E, Larsen T, Young H A, Reed D S, Geisbert J B, Scott D P, Kagan E, Jahrling P B and Davis K J (2003b) Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection *Am J Pathol* **163** 2347-2370
- Geisbert T W, Lee A C, Robbins M, Geisbert J B, Honko A N, Sood V *et al.* (2010) Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study *Lancet* **375** 1896-1905
- Geisbert T W, Young H A, Jahrling P B, Davis K J, Kagan E and Hensley L E (2003c) Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event *J Infect Dis* **188** 1618-1629
- Gire S K, Goba A, Andersen KG, Sealfon R S, Park D J, Kanneh L, Jalloh S *et al.* (2014) Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak *Science* **345** 1369-1372
- Goeijenbier M, van Kampen J J, Reusken C B, Koopmans M P and van Gorp E C (2014) Ebola virus disease: a review on epidemiology, symptoms, treatment and pathogenesis *Neth J Med* **72** 442-448
- Gordon S, Pluddemann A and Martinez Estrada F (2014) Macrophage heterogeneity in tissues: phenotypic diversity and functions *Immunol Rev* **262** 36-55
- Gregory S M, Harada E, Liang B, Delos S E, White J M and Tamm L K (2011) Structure and function of the complete internal fusion loop from Ebolavirus glycoprotein 2 *Proc Natl Acad Sci U S A* **108** 11211-11216
- Greninger A L, Naccache S N, Federman S, Yu G, Mbala P, Bres V *et al.* (2015) Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis *Genome Med* **7** 99
- Grolla A, Lucht A, Dick D, Strong J E and Feldmann H (2005) Laboratory diagnosis of Ebola and Marburg hemorrhagic fever *Bull Soc Pathol Exot* **98** 205-209
- Groseth A, Feldmann H and Strong J E (2007) The ecology of Ebola virus *Trends Microbiol* **15** 408-416
- Haas C N (2014) On the quarantine period for ebola virus *PLoS Curr* **14** 6
- Haasnoot J, de Vries W, Geutjes E J, Prins M, de Haan P and Berkhout B (2007) The Ebola virus VP35 protein is a suppressor of RNA silencing *PLoS Pathog* **3** e86
- Hacke M, Bjorkholm P, Hellwig A, Himmels P, de Almodovar C R, Brugger B, Wieland F and Ernst A M (2015) Inhibition of Ebola virus glycoprotein-mediated cytotoxicity by targeting its transmembrane domain and cholesterol *Nat Commun* **6** 7688
- Han Z, Boshra H, Sunyer J O, Zwiers S H, Paragas J and Harty

- R N (2003) Biochemical and functional characterization of the Ebola virus VP24 protein: implications for a role in virus assembly and budding *J Virol* **77**1793-1800
- Han Z, Licata J M, Paragas J and Harty R N (2007) Permeabilization of the plasma membrane by Ebola virus GP2 *Virus Genes* **34** 273-281
- Han Z, Madara J J, Herbert A, Prugar L I, Ruthel G, Lu J, Liu Y and Liu W (2015) Calcium regulation of hemorrhagic fever virus budding: mechanistic implications for host-oriented therapeutic intervention *PLoS Pathog* **11** e1005220
- Haque A, Hober D and Blondiaux J (2015) Addressing therapeutic options for Ebola virus infection in current and future outbreaks *Antimicrob Agents Chemother* **59** 5892-5902
- Hartlieb B, Muziol T, Weissenhorn W and Becker S (2007) Crystal structure of the C-terminal domain of Ebola virus VP30 reveals a role in transcription and nucleocapsid association *Proc Natl Acad Sci USA* **104** 624-629
- Hartlieb B and Weissenhorn W (2006) Filovirus assembly and budding *Virology* **344** 64-70
- Hartman A L, Bird B H, Towner J S, Antoniadou Z A, Zaki S R and Nichol S T (2008a) Inhibition of IRF-3 activation by VP35 is critical for the high level of virulence of ebola virus *J Virol* **82** 2699-2704
- Hartman A L, Ling L, Nichol S T and Hibberd M L (2008b) Whole-genome expression profiling reveals that inhibition of host innate immune response pathways by Ebola virus can be reversed by a single amino acid change in the VP35 protein *J Virol* **82** 5348-5358
- Heald A E, Iversen P L, Saoud J B, Sazani P, Charleston J S, Axtelle T *et al.* (2014) Safety and pharmacokinetic profiles of phosphorodiamidate morpholino oligomers with activity against ebola virus and marburg virus: results of two single-ascending-dose studies *Antimicrob Agents Chemother* **58** 6639-6647
- Henao-Restrepo A M, Longini I M, Egger M, Dean N E, Edmunds W J, Camacho A *et al.* (2015) Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial *Lancet* **386** 857-866
- Henao-Restrepo A M, Camacho A, Longini I M, Watson C H, Edmunds W J, Egger M *et al.* (2016) Efficacy and effectiveness of an rVSV- vectored vaccine in preventing Ebola virus disease: Final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ca Suffit !) *Lancet*: **6736** 32621:32626
- Hensley L E, Young H A, Jahrling P B and Geisbert T W (2002) Proinflammatory response during Ebola virus infection of primate models: Possible involvement of the tumor necrosis factor receptor superfamily *Immunol Lett* **80** 169-179
- Herbert A S, Davidson C, Kuehne A I, Bakken R, Braigen S Z, Gunn K E, Sean P. Whelan S P *et al.* (2015) Niemann-Pick C1 Is Essential for Ebolavirus Replication and Pathogenesis *In Vivo* **6**: e00565-15 doi: 10.1128/mBio.00565-1526
- Ho W E, Peh H Y, Chan T K and Wong W S (2014) Artemisinins: pharmacological actions beyond anti-malarial *Pharmacol Ther* **142** 126-139
- Hoenen T and Feldmann H (2014) Ebolavirus in West Africa, and the use of experimental therapies or vaccines *BMC Biol* **12** 80
- Hoenen T, Groseth A, Kolesnikova L, Theriault S, Ebihara H, Hartlieb B *et al.* (2006) Infection of naive target cells with virus-like particles: implications for the function of ebola virus VP24 *J Virol* **80** 7260-7264
- Hoenen T, Jung S, Herwig A, Groseth A and Becker S (2010) Both matrix proteins of Ebola virus contribute to the regulation of viral genome replication and transcription *Virology* **403** 56-66
- Hoenen T, Volchkov V, Kolesnikova L, Mittler E, Timmins J, Ottmann M, Reynard O, Becker S and Weissenhorn W (2005) VP40 octamers are essential for Ebola virus replication *J Virol* **79** 1898-1905
- Hood C L, Abraham J, Boyington J C, Leung K, Kwong P D and Nabel G J (2010) Biochemical and structural characterization of cathepsin L-processed Ebola virus glycoprotein: implications for viral entry and immunogenicity *J Virol* **84** 2972-2982
- Huang Y, Xu L, Sun Y and Nabel G J (2002) The assembly of Ebola virus nucleocapsid requires virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein *Mol Cell* **10** 307-316
- Hunt B J (2014) Bleeding and coagulopathies in critical care *N Engl J Med* **370** 847-859
- Hunt C L, Kolokoltsov A A, Davey R A and Maury W (2011) The Tyro3 receptor kinase Axl enhances macropinocytosis of Zaire ebolavirus *J Virol* **85** 334-347
- Hunt L, Gupta-Wright A, Simms V, Tamba F, Knott V, Tamba K, Heisenberg-Mansaray S *et al.* (2015) Clinical presentation, biochemical, and haematological parameters and their association with outcome in patients with Ebola virus disease: an observational cohort study *Lancet Infect Dis*. pii: S1473-3099(15)00144-9
- Ilinykh P A, Tigabu B, Ivanov A, Ammosova T, Obukhov Y, Garron T *et al.* (2014) Role of protein phosphatase 1 in dephosphorylation of Ebola virus VP30 protein and its

- targeting for the inhibition of viral transcription *J Biol Chem* **289** 22723-22738
- International Committee on Taxonomy of Viruses (2013 Release) Virus Taxonomy. Ebolavirus. <http://www.ictvonline.org/virusTaxonomy.asp>
- Iversen P L, Warren T K, Wells J B, Garza N L, Mourich D V, Welch L S, Panchal R G and Bavari S (2012) Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections *Viruses* **4** 2806-2830
- Jacobs M, Aarons E, Bhagani S, Buchanan R, Cropley I, Hopkins S et al. (2015) Post-exposure prophylaxis against Ebola virus disease with experimental antiviral agents: a case-series of health-care workers *Lancet Infect Dis* pii: S1473-3099(15)00228-5
- Jacobson S H, Yu G and Jokela J A (2016) A double-risk monitoring and movement restriction policy for Ebola entry screening at airports in the United States *Prev Med* **88** 33-38
- Jasenosky L D and Kawaoka Y (2004) Filovirus budding *Virus Res* **106** 181-188
- Jin H, Yan Z, Prabhakar B S, Feng Z, Ma Y, Verpooten D, Ganesh B and He B (2010) The VP35 protein of Ebola virus impairs dendritic cell maturation induced by virus and lipopolysaccharide *J Gen Virol* **91** 352-361
- Johansen L M, Brannan J M, Delos S E, Shoemaker C J, Stossel A, Lear C et al. (2013) FDA-approved selective estrogen receptor modulators inhibit Ebola virus infection *Sci Transl Med* **5**190ra79 doi: 10.1126/scitranslmed.3005471
- Johansen L M, DeWald L E, Shoemaker C J, Hoffstrom B G, Lear-Rooney C M, Stossel A et al. (2015) A screen of approved drugs and molecular probes identifies therapeutics with anti-Ebola virus activity *Sci Transl Med* **7** 290ra89
- Johnson R F, McCarthy S E, Godlewski P J and Harty R N (2006) Ebola virus VP35-VP40 interaction is sufficient for packaging 3E-5E minigenome RNA into virus-like particles *J Virol* **80** 5135-5144
- Johnson J C, Martinez O, Honko A N, Hensley L E, Olinger G G and Basler C F (2014) Pyridinyl imidazole inhibitors of p38 MAP kinase impair viral entry and reduce cytokine induction by Zaire ebolavirus in human dendritic cells *Antiviral Res* **107** 102-109
- Judson S et al., (2015) Understanding Ebola virus transmission *Viruses* **7** 511-521
- Jun S R, Leuze M R, Nookaew I, Uberbacher E C, Land M, Zhang Q et al. (2015) Ebolavirus comparative genomics *FEMS Microbiol Rev* **39** 764-778
- Kaletsky R L, Francica J R, Agrawal-Gamse C and Bates P (2009) Tetherin-mediated restriction of filovirus budding is antagonized by the Ebola glycoprotein *Proc Natl Acad Sci USA* **106** 2886-2891
- Ker K, Tansley G, Beecher D, Perner A, Shakur H, Harris T and Roberts I (2015) Comparison of routes for achieving parenteral access with a focus on the management of patients with Ebola virus disease *Cochrane Database Syst Rev* **2** DOI: 10.1002/14651858
- Kesel A J, Huang Z, Murray M G, Prichard M N, Caboni L, Nevin D K et al. (2014) Retinazone inhibits certain blood-borne human viruses including Ebola virus Zaire *Antivir Chem Chemother* **23** 197-215
- Kibuuka H, Berkowitz N M, Millard M, Enama M E, Tindikahwa A, Sekiziyivu A B, Costner P et al. (2014) Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial *Lancet* S0140-6736 62385-0 doi: 10.1016/S0140-6736(14)62385-0
- Kilgore P E, Grabenstein J D, Salim A M and Rybak M (2015) Treatment of ebola virus disease *Pharmacotherapy* **35** 43-53
- Kimberlin D W, Acosta E P, Sánchez P J, Sood S, Agrawal V, Homans J et al. (2008) Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease *J Infect Dis* **197** 836-845
- Kimberlin C R, Bornholdt Z A, Li S, Woods V L Jr., MacRae I J and Saphire E O (2010) Ebolavirus VP35 uses a bimodal strategy to bind dsRNA for innate immune suppression *Proc Natl Acad Sci USA* **107** 314-319
- Kiraly L N, Differding J A, Enomoto T M, Sawai R S, Muller P J, Diggs B et al. (2006) Resuscitation with normal saline (NS) vs. lactated ringers (LR) modulates hypercoagulability and leads to increased blood loss in an uncontrolled hemorrhagic shock swine model *J Trauma* **61** 57-64
- Kirchdoerfer R N, Abelson D M, Li S, Wood M R and Saphire E O (2015) Assembly of the Ebola virus nucleoprotein from a chaperoned VP35 complex *Cell Rep* **12** 140-149
- Klenk H D and Feldmann H (2004) Ebola and Marburg Viruses: Molecular and Cellular Biology, Wymondham: Horizon Bioscience.
- Kolokoltsov A A, Adhikary S, Garver J, Johnson L, Davey R A and Vela E M (2012) Inhibition of Lassa virus and Ebola virus infection in host cells treated with the kinase inhibitors genistein and tyrphostin *Arch Virol* **157** 121-127

- Kortepeter M G, Bausch DG and Bray M (2011) Basic clinical and laboratory features of filoviral hemorrhagic fever *J Infect Dis* **204** Suppl 3 S810-816
- Kouznetsova J, Sun W, Martinez-Romero C, Tawa G, Shinn P and Chen C Z (2014) Schimmer <http://www.nature.com/emi/journal/v3/n12/full/emi201488a.html> - aff4 A, Sanderson, P., McKew, J. C., Zheng, W., García-Sastr, A., Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs. *Emerging Microbes & Infections* **3**: e84
- Ksiazek T G, West C P, Rollin P E, Jahrling P B and Peters C J (1999) ELISA for the detection of antibodies to Ebola viruses *J Infect Dis* **179** S192-198
- Kuhl A and Pohlmann S (2012) How Ebola virus counters the interferon system *Zoonoses Public Health* **59** 116-131
- Kuhn J H, Andersen KG, Baize S *et al.* (2014) Nomenclature- and database-compatible names for the two Ebola virus variants that emerged in Guinea and the Democratic Republic of the Congo in 2014 *Viruses* **6** 4760-4799
- Kuhn J H, Becker S, Ebihara H, Geisbert T W, Johnson K M, Kawaoka Y *et al.* (2010) Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and viruses, and virus abbreviations *Archives of Virology* **155** 2083-2103
- Kugelman J R, Kugelman-Tonos J, Ladner J T, Pettit J, Keeton C M, Nagle E R *et al.* (2015) Emergence of Ebola virus escape variants in infected nonhuman primates treated with the MB-003 antibody cocktail *Cell Rep* **12** 2111-2120
- Kumar C V M N and Gopal D V R S (2014) Possible risk of Ebola out break in India: how well are we prepared? *Current Sci* **107** 937-937
- Ledgerwood J E, DeZure A D, Stanley D A, Novik L, Enama M E, Berkowitz N M, Hu Z *et al.* (2014) Chimpanzee adenovirus vector Ebola vaccine - preliminary report *N Engl J Med* DOI: 10.1056/NEJMoa1410863
- Lee J E, Fusco M L, Hessel A J, Oswald W B, Burton D R and Saphire E O (2008) Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor *Nature* **454** 177-182
- Lennemann N J, Rhein B A, Ndungo E, Chandran K, Qiu X and Maury W (2014) Comprehensive functional analysis of N-linked glycans on Ebola virus GP1 *MBio* **5** e00862-13
- Leroy E M, Baize S, Volchkov V E, Fisher-Hoch S P, Georges-Courbot M C, Lansoud-Soukate J *et al.* (2000) Human asymptomatic Ebola infection and strong inflammatory response *Lancet* **355** 2210-2215
- Leroy E M, Becquart P, Wauquier N and Baize S (2011a) Evidence for Ebola virus super antigen activity *J Virol* **85** 4041-4042
- Leroy E M, Gonzalez J P and Baize S (2011b) Ebola and Marburg haemorrhagic fever viruses: major scientific advances, but a relatively minor public health threat for Africa *Clin Microbiol Infect* **17** 964-976
- Leroy E M, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P *et al.* (2005) Fruit bats as reservoirs of Ebola virus *Nature* **438** 575-576
- Leroy E M, Rouquet P, Formenty P, Souquiere S, Kilbourne A, Froment J M, Bermejo M *et al.* (2004) Multiple Ebola virus transmission events and rapid decline of central African wildlife *Science* **303** 387-390
- Leung D W, Borek D, Basler C F and Amarasinghe G K (2015) An intrinsically disordered peptide from Ebola Virus VP35 controls viral RNA synthesis by modulating nucleoprotein-RNA interactions *Cell Reports* <http://dx.doi.org/10.1016/j.celrep.2015.03.034>
- Leung D W, Prins K C, Borek D M, Farahbakhsh M, Tufariello J M, Ramanan P, Nix J C and Helgeson L A (2010) Structural basis for dsRNA recognition and interferon antagonism by Ebola VP35 *Nat Struct Mol Biol* **17** 165-172
- Levi M, Schultz M and van der Poll T (2010) Disseminated intravascular coagulation in infectious disease *Semin Thromb Hemost* **36** 367-377
- Li Y H and Chen S P (2014) Evolutionary history of Ebola virus *Epidemiol Infect* **142** 1138-1145
- Liang J, Sagum C A, Bedford M T, Sidhu S S, Sudol M, Han Z *et al.* (2017) Chaperone-mediated autophagy protein BAG3 negatively regulates Ebola and Marburg VP40-mediated egress *PLOS Path* **13** e 1006132
- Licata J M, Johnson R F, Han Z and Harty R N (2004) Contribution of ebola virus glycoprotein, nucleoprotein, and VP24 to budding of VP40 virus-like particles *J Virol* **78** 7344-7351
- Litterman N, Lipinski C and Ekins S (2015) Small molecules with antiviral activity against the Ebola virus. F1000Res **4** 38
- Liu S, Cai X, Wu J, Cong Q, Chen X, Li T, Du F, Ren J, Wu Y, Grishin N and Chen Z J (2015) Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation *Science* pii: aaa2630
- Liu Y, Cocka L, Okumura A, Zhang Y A, Sunyer J O and Harty R N (2010) Conserved motifs within Ebola and Marburg virus VP40 proteins are important for stability, localization, and subsequent budding of virus-like particles *J Virol* **84** 2294-2303

- Liu G, Zhao S, Bailey JA, Sahinalp S C, Alkan C, Tuzun E, Green E D and Eichler E E (2003) Analysis of primate genomic variation reveals a repeat-driven expansion of the human genome *Genome Res* **13** 358-368
- Liu X, Speranza E, Munoz-Fontela C, Haldenby S, Rickett N Y et al. (2017) Transcriptional signatures differentiate survival from fatal outcomes in humans infected with Ebola virus *Genome Biol* **18** 4
- Longdon B, Hadfield J D, Day J P, Smith S C, McGonigle J E, Cogni R, Cao C and Jiggins F M (2015) The causes and consequences of changes in virulence following pathogen host shifts *PLoS Pathog* **11** e1004728
- Lucht A, Grunow R, Moller P, Feldmann H and Becker S (2003) Development, characterization and use of monoclonal VP40-antibodies for the detection of Ebola virus *J Virol Methods* **111** 21-28
- Luczkowiak J, Munoz A, Sanchez-Navarro M, Ribeiro-Viana R, Ginieis A, Illescas B M, Martin N, Delgado R and Rojo J (2013) Glycofullerenes inhibit viral infection *Biomacromolecules* **14** 431-437
- Luthra P, Ramanan P, Mire C E, Weisend C, Tsuda Y, Yen B et al. (2013) Mutual antagonism between the Ebola virus VP35 protein and the RIG-I activator PACT determines infection outcome *Cell Host Microbe* **14** 74-84
- Macleod C (2010) Towards a philosophical account of crimes against humanity *Eur J Int Law* **21** 281-302
- Madrid P B, Chopra S, Manger I D, Gilfillan L, Keepers T R, Shurtleff A C, Green C E et al. (2013) A systematic screen of FDA-approved drugs for inhibitors of biological threat agents *PLoS One* **8** e60579
- Mahanty S and Bray M (2004) Pathogenesis of filoviral haemorrhagic fevers *Lancet Infect Dis* **4** 487-498
- Mahanty S, Hutchinson K, Agarwal S, McRae M, Rollin P E and Pulendran B (2003) Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses *J Immunol* **170** 2797-2801
- Manicassamy B, Wang J, Jiang H and Rong L (2005) Comprehensive analysis of ebola virus GP1 in viral entry *J Virol* **79** 4793-4805
- Martinez O, Valmas C and Basler C F (2007) Ebola virus-like particle-induced activation of NF- κ B and Erk signaling in human dendritic cells requires the glycoprotein mucin domain *Virology* **364** 342-354
- Marzi A, Akhavan A, Simmons G, Gramberg T, Hofmann H, Bates P, Lingappa V R and Pohlmann S (2006a) The signal peptide of the ebolavirus glycoprotein influences interaction with the cellular lectins DC-SIGN and DC-SIGNR *J Virol* **80** 6305-6317
- Marzi A, Ebihara H, Callison J, Groseth A, Williams K J, Geisbert T W and Feldmann H (2011a) Vesicular stomatitis virus-based Ebola vaccines with improved cross-protective efficacy *J Infect Dis* **204** S1066-1074
- Marzi A, Engelmann F, Feldmann F, Haberthur K, Shupert W L, Brining D et al. (2013) Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates *Proc Natl Acad Sci USA* **110** 1893-1898
- Marzi A and Feldmann H (2014) Ebola virus vaccines: an overview of current approaches *Expert Rev Vaccines* **13** 521-531
- Marzi A, Feldmann F, Geisbert T W, Feldmann H and David Safronetz D (2015) Vesicular stomatitis virus-based vaccines against Lassa and Ebola viruses *Emerg Infect Dis* **21** 305-307
- Marzi A, Halfmann P, Hill-Batorski L, Feldmann F, Shupert W L, Neumann G, Feldmann H and Kawaoka Y (2015b) An Ebola whole-virus vaccine is protective in nonhuman primates *Science* DOI: 10.1126/science.aaa4919
- Marzi A, Murphy A A, Feldmann F, Parkins C J, Haddock E, Hanley P W, Emery M J, Engelmann F et al. (2016) Cytomegalovirus-based vaccine expressing Ebola virus glycoprotein protects nonhuman primates from Ebola virus infection *Sci Rep* **6** 21674
- Marzi A, Robertson S J, Haddock E, Feldmann F, Hanley P W, Scott D P et al. (2015) EBOLA VACCINE. VSV-EBOV rapidly protects macaques against infection with the 2014/15 Ebola virus outbreak strain *Science* **349** 739-742
- Marzi A, Wegele A and Pohlmann S (2006b) Modulation of virion incorporation of Ebolavirus glycoprotein: effects on attachment, cellular entry and neutralization *Virology* **352** 345-356
- Mate S E, Kugelman J R, Nyenswah T G, Ladner J T, Wiley M R, Cordier-Lassalle T et al. (2015) Molecular evidence of sexual transmission of Ebola virus *N Engl J Med* DOI: 10.1056/NEJMoa1509773
- Mateo M, St. Reid P, Leung L W, Basler C F and Volchkov V E (2009) Ebolavirus VP24 binding to karyopherins is required for inhibition of interferon signaling *J Virol* **84** 1169-1175
- Mateo M, Carbonnelle C, Reynard O, Kolesnikova L, Nemirov K, Page A, Volchkova V A and Volchkov V E (2011) VP24 is a molecular determinant of Ebola virus virulence in guinea pigs *J Infect Dis* **204** S1011-20
- Maughan R (2014) Ignorance and bush meat trade cause of Ebola epidemic *The Wild Life News* (www.wildlifeneeds.com/2014/07/25/)

- McElroy A K, Akondy R S, Davis C W, Ellebedy A H, Mehta A K, Kraft C S *et al.* (2015) Human Ebola virus infection results in substantial immune activation *Proc Natl Acad Sci USA* **112** 4719-4724
- Mehedi M, Falzarano D, Seebach J, Hu X, Carpenter M S, Schnittler H J and Feldmann H (2011) A new Ebola virus nonstructural glycoprotein expressed through RNA editing *J Virol* **85** 5406-5414
- Mehedi M, Hoenen T, Robertson S, Ricklefs S, Dolan M A, Taylor T, Falzarano D, Ebihara H, Porcella S F and Feldmann H (2013) Ebola virus RNA editing depends on the primary editing site sequence and an upstream secondary structure *PLoS Pathog* **9** e1003677
- Mehta A, Zitzmann N, Rudd P M, Block T M and Dwek R A (1998) Alpha-glucosidase inhibitors as potential broad based anti-viral agents *FEBS Lett.* **430** 17-22
- Meyer M, Garron T, Lubaki N M, Mire C E, Fenton K A, Klages C, Olinger G G, Geisbert T W, Collins P L and Bukreyev A (2015) Aerosolized Ebola vaccine protects primates and elicits lung-resident T cell responses *J Clin Invest* **125** 3241-3255
- Messaoudi I, Amarasinghe G K and Basler C F (2015) Filovirus pathogenesis and immune evasion: insights from Ebola virus and Marburg virus *Nat Rev* **13** 1-13
- Miller M E, Adhikary S, Kolokoltsov A A and Davey R A (2012a) Ebolavirus requires acid sphingomyelinase activity and plasma membrane sphingomyelin for infection *J Virol* **86** 7473-7483
- Miller E H and Chandran K (2012) Filovirus entry into cells - new insights *Curr Opin Virol* **2** 206-214
- Miller E H, Obernosterer G, Raaben M, Herbert A S, Deffieu M S, Krishnan A, Ndungo E *et al.* (2012b) Ebola virus entry requires the host-programmed recognition of an intracellular receptor *EMBO J* **31** 1947-1960
- Mingo R M, Simmons J A, Shoemaker C J, Nelson E A, Schornberg K L, D'Souza R S, Casanova J E and White J M (2015) Ebola virus and severe acute respiratory syndrome coronavirus display late cell entry kinetics: evidence that transport to NPC1⁺ endolysosomes is a rate-defining step *J Virol* **89** 2931-2943
- Mire C E, Matassov D, Geisbert J B, Latham T E, Agans K N, Xu R *et al.* (2015) Single-dose attenuated Vesiculovax vaccines protect primates against Ebola Makona virus *Nature* doi:10.1038/nature14428
- Misasi J, Gilman M S, Kanekiyo M, Gui M, Cagigi A, Mulangu S, Corti D *et al.* (2016) Structural and molecular basis for Ebola virus neutralization by protective human antibodies *Science* **351** 1343-1346
- Mohan G S, Li W, Ye L, Compans, R W and Yang C (2012) Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus *PLoS Pathog* **8** e1003065
- Mohan G S, Ye L, Li W, Monteiro A, Lin X, Sapkota B, Pollack B P, Compans R W and Yang C (2015) Less is more: ebola virus surface glycoprotein expression levels regulate virus production and infectivity *J Virol* **89** 1205-1217
- Mohamadzadeh M, Chen L and Schmaljohn A L (2007) How Ebola and Marburg viruses battle the immune system *Nat Rev Immunol* **7** 556-567
- Moller-Tank S, Kondratowicz A S, Davey R A, Rennert P D and Maury W (2013) Role of the phosphatidyserine receptor TIM-1 in enveloped-virus entry *J Virol* **87** 8327-8341
- Moreau M, Spencer C, Gozalbes J, Colebunders R, Lefevre A, Gryseels S, Borremans B *et al.* (2015) Lactating mothers infected with Ebola virus: EBOV RT-PCR of blood only may be insufficient *Euro Surveil* **20** pii: 21017
- Morvan J M, Deubel V, Gounon P, Nakoune E, Barriere P, Murri S, Perpete O *et al.* (1999) Identification of Ebola virus sequences present as RNA or DNA in organs of terrestrial small mammals of the Central African Republic *Microbes Infect* **1** 1193-1201
- Muhlberger E (2007) Filovirus replication and transcription *Future Virol* **2** 205-215
- Muhlberger E, Weik M, Volchkov V E, Klenk H D and Becker S (1999) Comparison of the transcription and replication strategies of Marburg virus and Ebola virus by using artificial replication systems *J Virol* **73** 2333-2342
- Muñoz A, Sigwalt D, Illescas B M, Luczkowiak J, Rodríguez-Pérez L, Nierengarten I *et al.* (2016) Synthesis of giant globular multivalent glycofullerenes as potent inhibitors in a model of Ebola virus infection *Nat Chem* **8** 50-57
- Murin C D, Fusco M L, Bornholdt Z A, Qiu X, Olinger G G, Zeitlin L, Kobinger G P, Ward A B and Saphire E O (2014) Structures of protective antibodies reveal sites of vulnerability on Ebola virus *Proc Natl Acad Sci USA* **111** 17182-17187
- Murphy A A, Redwood A J, Jarvis M A (2016) Self-disseminating vaccines for emerging infectious diseases *Expert Rev Vaccines* **15** 31-39
- Nanbo A, Imai M, Watanabe S, Noda T, Takahashi K, Neumann G, Halfmann P and Kawaoka Y (2010) Ebolavirus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner *PLoS Pathog* **6** e1001121
- Napier R J, Norris B A, Swimm A, Giver C R, Harris W A, Laval J, Napier B A, Patel G, Crump R, Peng Z *et al.* (2015) Low doses of imatinib induce myelopoiesis and enhance

- host anti-microbial immunity *PLoS Pathog* **11** e1004770
- Naresh Kumar C V M and Sai Gopal D V R (2014) Possible risk of Ebola outbreak in India: how well are we prepared? *Curr Sci* **107** 937
- Natesan M, Jensen S M, Keeasey S L, Kamata T, Kuehne A I, Stonier S *et al.* (2016) Human survivors of disease outbreaks caused by Ebola or Marburg viruses exhibit cross-reactive and long-lived antibody response. *Clin Vaccine Immunol* **23** 717-724
- Ng M, Ndungo E, Kaczmarek M E, Herbert A S, Binger T, Kuehne A I, Jangra R K *et al.* (2015) Filovirus receptor NPC1 contributes to species-specific patterns of ebolavirus susceptibility in bats *Elife* **4** e11785
- Nierengarten I and Nierengarten J F (2014) Fullerene sugar balls: a new class of biologically active fullerene derivatives *Chem Asian J* **9** 1436-1444
- Niikura M, Ikegami T, Saijo M, Kurane I, Miranda M E and Morikawa S (2001) Detection of Ebola viral antigen by enzyme-linked immunosorbent assay using a novel monoclonal antibody to nucleoprotein *J Clin Microbiol* **39** 3267-3271
- Noda T, Sagara H, Suzuki E, Takada A, Kida H and Kawaoka Y (2002) Ebola virus VP40 drives the formation of virus-like filamentous particles along with GP *J Virol* **76** 4855-4865
- Noda T, Watanabe S, Sagara H and Kawaoka Y (2007) Mapping of the VP40-binding regions of the nucleoprotein of Ebola virus *J Virol* **81** 3554-3562
- O'Brien L M, Stokes M G, Lonsdale S G, Maslowski D R, Smither S J, Lever M S, Laws T R and Perkins S D (2014) Vaccination with recombinant adenoviruses expressing Ebola virus glycoprotein elicits protection in the interferon alpha/beta receptor knock-out mouse *Virology* **453** 324-333
- Oestereich L, Ludtke A, Wurr S, Rieger T, Munoz-Fontela C and Gunther S (2014) Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model *Antiviral Res* **105** 17-21
- O'Keefe B R, Vojdani F, Buffa V, Shattock R J, Montefiori D C, Bakke J *et al.* (2009) Scaleable manufacture of HIV-1 entry inhibitor griffithsin and validation of its safety and efficacy as a topical microbicide component *Proc Natl Acad Sci USA* **106** 6099-6104
- Olejnik J, Ryabchikova E, Corley R B and Muhlberger E (2011) Intracellular events and cell fate in filovirus infection *Viruses* **3** 1501-1531
- Olinger G G Jr, Pettitt J, Kim D, Working C, Bohorov O, Bratcher B, Hiatt E, Hume S D, Johnson A K *et al.* (2012) Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques *Proc Natl Acad Sci USA* **109** 18030-18035
- Olival K J and Hayman D T (2014) Filoviruses in bats: current knowledge and future directions *Viruses* **6** 1759-1788
- Olival K J, Islam A, Yu M, Anthony S J, Epstein J H, Khan S A *et al.* (2013) Ebola virus antibodies in fruit bats, Bangladesh *Emerg Infect Dis* **19** 270-273
- Olsen M E, Filone C M, Rozelle D, Mire C E, Agans K N, Hensely L and Connor J H (2016) Polyamines and hypusination are required for Ebola virus gene expression and replication *mBio* **7**: e00882-16
- Pallesen J, Murin C D, deVal N, Cottree C A, Hastie K M, Turner H L *et al.* (2016) Structures of Ebola virus GP and sGP in complex with therapeutic antibodies *Nature Microbiol* **1** 16128
- Pang Z, Li A, Li J, Qu J, He C *et al.* (2014) Comprehensive multiplex one-step real-time TaqMan qRT-PCR assays for detection and quantification of hemorrhagic fever viruses *PLoS ONE* **9** e95635
- Park D J, Dudas G, Wohl S, Goba A, Whitmer S L, Andersen K G *et al.* (2015) Ebola virus epidemiology, transmission and evolution during seven months in Sierra Leone *Cell* **161** 1516-1526
- Patel J M, Snaith C, Thickett D R, Linhartova L, Melody T, Hawkey P, Barnett A H, Jones A, Hong T, Cooke M W, Perkins G D and Gao F (2012) Randomized double-blind placebo-controlled trial of 40 mg/day of atorvastatin in reducing the severity of sepsis in ward patients (ASEPSIS Trial) *Crit Care* **16** R231
- Peterson A T, Carroll D and Mills J N (2004) Potential mammalian filovirus reservoirs *Emerg Infect Dis* **10** 2073-2081
- Peterson A T, Papes M, Carroll D S, Leirs H and Johnson K M (2007) Mammal taxa constituting potential coevolved reservoirs of filoviruses *J Mammal* **88** 1544-1554
- Pattabhi S, Wilkins C R, Dong R, Knoll M L, Posakony J, Kaiser S, Mire C E *et al.* (2015) Targeting Innate Immunity for Antiviral Therapy through Small Molecule Agonists of the RLR Pathway *J Virol* **90** 2372-2387
- Pourrut X, Delicat A, Rollin P E, Ksiazek T G, Gonzalez J P and Leroy E M (2007) Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species *J Infect Dis* **196** S176-183
- Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Delicat A, Yaba P, Nkoghe D, Gonzalez J P and Leroy E M (2005) The natural history of Ebola virus in Africa *Microbes Infect* **7** 1005-1014
- Prescott J, Trenton Bushmaker T, Fischer R, Miazgowiec K, Judson S and Munster V J (2015) Postmortem stability of

- Ebola virus *Emerg Infect Dis* DOI: 10.3201/eid2105.150041
- Prins K C, Cardenas W B and Basler C F (2009) Ebola Virus Protein VP35 impairs the function of interferon regulatory factor-activating kinases IKK and TBK-1 *J Virol* **83** 3069-3077
- Public Health Agency of Canada (2014) Pathogen safety data sheets. Ebolavirus. Public Health Agency of Canada, Ottawa, Canada. <http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/ebola-arg.php>
- Qiu X, Audet J, Wong G, Pillet S, Bello A, Cabral T, Strong J E, Plummer F, Corbett C R, Alimonti J B and Kobinger G P (2012) Successful treatment of ebola virus-infected cynomolgus macaques with monoclonal antibodies *Sci Transl Med* **4** 138ra81
- Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti J B and Fausther-Bovendo H (2014) Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature* **514** 47-53
- Radoshitzky S R, Dong L, Chi X, Clester J C, Retterer C, Spurgers K *et al.* (2010) Infectious Lassa virus, but not filoviruses, is restricted by BST-2/tetherin *J Virol* **84** 10569-10580
- Radoshitzky S R, Warfield K L, Chi X, Dong L, Kota K, Bradfute S B, Gearhart J D *et al.* (2011) Ebolavirus delta-peptide immunoadhesins inhibit marburgvirus and ebolavirus cell entry *J Virol* **85** 8502-8513
- Ramanan P, Shabman R S, Brown C S, Amarasinghe G K, Basler C F, Leung D W (2011) Filoviral immune evasion mechanisms *Viruses* **3** 1634-1649
- Rampling T, Ewer K, Bowyer G, Wright D, Imoukhuede E B, Payne R, Hartnell F, Gibani M, Bliss C, Minhinnick A *et al.* (2015) A Monovalent Chimpanzee Adenovirus Ebola Vaccine - Preliminary Report *N Engl J Med* DOI: 10.1056/NEJMoa1411627
- Rasmussen A L, Okumura A, Ferris M T, Green R, Feldmann F, Kelly S M, Scott D P *et al.* (2014) Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance *Science* **346** 987-991
- Rebsburg S, Helfer M, Schneider M, Koppensteiner H, Eberle J, Schindler M *et al.* (2016) Potent in vitro antiviral activity of *Cistus incanus* extract against HIV and Filoviruses targets viral envelope proteins *Sci Rep* **6** 20394
- Reed D S, Hensley L E, Geisbert J B, Jahrling P B and Geisbert T W (2004) Depletion of peripheral blood T lymphocytes and NK cells during the course of ebola hemorrhagic Fever in cynomolgus macaques *Viral Immunol* **17** 390-400
- Reed P E, Mulangu S, Cameron K N, Ondzie A U, Joly D, Bermejo M, Rouquet P *et al.* (2014) A new approach for monitoring ebolavirus in wild great apes *PLoS Negl Trop Dis* **8** e3143
- Regules J A, Beigel J H, Paolino K M, Voell J, Castellano A R, Munoz P *et al.* (2015) A recombinant vesicular stomatitis virus Ebola vaccine - preliminary report *N Engl J Med* DOI: 10.1056/NEJMoa1414216
- Reid S P, Leun L W, Hartman A L, Martinez O, Shaw M L, Carbonnelle C, Volchkov V E, Nichol S T and Basler C F (2006) Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation *J Virol* **80** 5156-5167
- Reid S P, Valmas C, Martinez O, Sanchez F M and Basler C F (2007) Ebola virus VP24 proteins inhibit the interaction of NPI-1 subfamily karyopherin alpha proteins with activated STAT1 *J Virol* **81** 13469-13477
- Reiter P, Turell M, Coleman R, Miller B, Maupin G, Liz J, Kuehne A *et al.* (1999) Field investigations of an outbreak of Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: arthropod studies *J Infect Dis* **179** S148-54
- Rhein B A and Maury W J (2015) Ebola virus entry into host cells: identifying therapeutic strategies *Virology* **2** 115-124
- Rhein B A, Powers L S, Rogers K, Anantpadma M, Singh B K, Sakurai Y, Bair T *et al.* (2015) Interferon- α inhibits Ebola virus infection *PLoS Pathog* **11** e1005263
- Richardson E T, Kelly J D, Barrie M B, Mesman A W, Karku S, Quiwa K *et al.* (2016) Minimally symptomatic infection in an Ebola 'Hotspot': A cross-sectional survey. *PLoS Neglect Trop Dis* **10** e005087
- Roberts I and Perner A (2014) Ebola virus disease: clinical care and patient-centred research *The Lancet* **384** 2001-2002
- Roddy P, Howard N, Van Kerkhove M D, Lutwama J, Wamala J, Yoti Z, Colebunders R *et al.* (2012) Clinical manifestations and case management of Ebola haemorrhagic fever caused by a newly identified virus strain, Bundibugyo, Uganda, 2007-2008 *PLoS One* **7** e52986
- Rollin P E, Bausch D G and Sanchez A (2007) Blood chemistry measurements and D-Dimer levels associated with fatal and nonfatal outcomes in humans infected with Sudan Ebola virus *J Infect Dis* **196** S364-371
- Rowe A K, Bertolli J, Khan A S, Mukunu R, Muyembe-Tamfum J J, Bressler D, Williams A J, Peters C J *et al.* (1999) Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidémies à Kikwit *J Infect Dis* **179** S28-35
- Ruf W (2004) Emerging roles of tissue factor in viral hemorrhagic fever *Trends Immunol* **25** 461-464

- Ryabchikova E I, Kolesnikova L V and Luchko S V (1999) An analysis of features of pathogenesis in two animal models of Ebola virus infection *J Infect Dis* **179** S199-202
- Saeed M F, Kolokoltsov A A, Albrecht T and Davey R A (2010) Cellular entry of Ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes *PLoS Pathog* **6** e1001110
- Sadler A J and Williams B R (2008) Interferon-inducible antiviral effectors *Nat Rev Immunol* **8** 559-568
- Sakurai Y, Kolokoltsov A A, Chen C C, Tidwell M W, Bauta W E, Klugbauer N, Grimm C, Wahl-Schott C, Biel M and Davey R A (2015) Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment *Science* **347** 995-998
- Sanchez A (2001) Filoviridae: Marburg and Ebola Viruses. In D. M. Knipe, & P. M. Howley (Eds.), *Fields virology* (4th ed., pp. 1279-1304). Philadelphia, PA.: Lippincott-Ravenpp.
- Sanchez A, Geisbert T W and Feldmann H (2006) Filoviridae: Marburg and Ebola viruses. In: Knipe DM, Howley PM, editors. *Fields virology*. Philadelphia: Lippincott Williams & Wilkins; 1409-1448
- Sanchez A, Lukwiya M, Bausch D, Mahanty S, Sanchez A J, Wagoner K D and Rollin P E (2004) Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: cellular responses, virus load, and nitric oxide levels *J Virol* **78** 10370-10377
- Sanchez A, Trappier S G, Mahy B W, Peters C J and Nichol S T (1996) The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing *Proc Natl Acad Sci USA* **93** 3602-3607
- Sanchez A, Wagoner K E and Rollin P E (2007) Sequence-based human leukocyte antigen-B typing of patients infected with Ebola virus in Uganda in 2000: identification of alleles associated with fatal and nonfatal disease outcomes *J Infect Dis* **196** S329-336
- Sarwar U N, Costner P, Enama M E, Berkowitz N, Hu Z, Hendel C S, Sitar S, Plummer S *et al.* (2015) Safety and immunogenicity of DNA vaccines encoding ebolavirus and marburgvirus wild-type glycoproteins in a phase I clinical trial *J Infect Dis* **211** 549-557
- Schindler C, Levy D E and Decker T (2007) JAK-STAT signaling: from interferons to cytokines *J Biol Chem* **282** 20059-20063
- Schoggins J W, Wilson S J, Panis M, Murphy M Y, Jones C T, Bieniasz P and Rice C M (2011) A diverse range of gene products are effectors of the type I interferon antiviral response *Nature* **472** 481-485
- Schumann M, Gantke T and Muhlberger E (2009) Ebola virus VP35 antagonizes PKR activity through its C-terminal interferon inhibitory domain *J Virol* **83** 8993-8997
- Scott J T, Sesay F R, Massaquoi T A, Idriss B R, Sahr F and Semple M G (2016) Post-Ebola Syndrome, Sierra Leone *Emerg Infect Dis* **22** 641-646
- Semper A E, Broadhurst M J, Richards J, Foster G M, Simpson A J and Logue C H (2016) Performance of the GeneXpert Ebola Assay for diagnosis of Ebola virus disease in Sierra Leone: A Field Evaluation Study *PLoS Med* **13** e1001980
- Shabman R S, Jabado O J, Mire C E, Stockwell T B, Edwards M, Mahajan M, Geisbert T W and Basler C F (2014) Deep sequencing identifies noncanonical editing of Ebola and Marburg virus RNAs in infected cells *M Bio* **5** e02011
- Sheng M, Zhong Y, Chen Y, Du J, Ju X, Zhao C, Zhang G, Zhang L, Liu K, Yang N *et al.* (2014) Hsa-miR-1246, hsa-miR-320a and hsa-miR-196b-5p inhibitors can reduce the cytotoxicity of Ebola virus glycoprotein in vitro *Sci China Life Sci* **57** 959-972
- Shoemaker C J, Schornberg K L, Delos S E, Scully C, Pajouhesh H, Olinger G G, Johansen L M and White J M (2013) Multiple cationic amphiphiles induce a Niemann-Pick C phenotype and inhibit Ebola virus entry and infection *PLoS One* **8** e56265
- Shuchman M (2014) Could interferon help treat Ebola? *CMAJ* **186** doi: 10.1503/cmaj.109-4906
- Sissoko D, Laouenan C, Folkesson E, M'Lebing A B, Beavogui A H, Baize S, Camara A M *et al.* (2016) Experimental Treatment with Favipiravir for Ebola Virus Disease (the JIKI Trial): A Historically Controlled, Single-Arm Proof-of-Concept Trial in Guinea *PLoS Med* **13** e1001967
- Skalsky R L and Cullen B R (2010) Viruses, microRNAs, and host interactions *Annu Rev Microbiol* **64** 123-141
- Smith T C (2010) Ebola. *Deadly Diseases and Epidemic Series*. Chelsea House: Philadelphia, PA 2005 (Second edition released November 2010)
- Smither S J, Eastaugh L S, Steward J A, Nelson M, Lenk R P and Lever M S (2014) Post-exposure efficacy of oral T-705 (Favipiravir) against inhalational Ebola virus infection in a mouse model *Antiviral Res* **104** 153-155
- Soni S P, Adu-Gyamfi E, Yong S S, Jee C S and Stahelin R V (2013) The Ebola virus matrix protein deeply penetrates the plasma membrane: an important step in viral egress *Biophys J* **104** 1940-1949
- Soni S P and Stahelin R V (2014) The Ebola virus matrix protein

- VP40 selectively induces vesiculation from phosphatidylserine-enriched membranes *J Biol Chem* **289** 33590-33597
- Spence J S, Krause T B, Mittler E, Jangra R K and Chandran K (2016) Direct visualization of Ebola virus fusion triggering in the endocytic pathway *MBio* **7** pii: e01857-15
- Spickler A R (2014) Ebolavirus and Marburgvirus Infections. The Centre for Food Security and Public Health. IOWA State University, Institute for International Cooperation in Animal Biologics
- Stanley D A, Honko A N, Asiedu C, Trefry J C, Lau-Kilby AW, Johnson J C, Hensley L *et al.* (2014) Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge *Nat Med* **20** 1126-1129
- Stephens K W, Hutchins R J and Dauphin L A (2010) Cross-platform evaluation of commercial real-time reverse transcription PCR master mix kits using a quantitative 5' nuclease assay for Ebola virus *Mol Cell Probes* **24** 370-375
- Subbotina E, Dadaeva A, Kachko A and Chepurnov A (2010) Genetic factors of Ebola virus virulence in guinea pigs *Virus Res* **153** 121-133
- Sullivan N, Yang Z Y and Nabel G J (2003) Ebola virus pathogenesis: implications for vaccines and therapies *J Virol* **77** 9733-9737
- Sullivan N J, Peterson M, Yang Z Y, Kong W P, Duckers H, Nabel E and Nabel G J (2005) Ebola virus glycoprotein toxicity is mediated by a dynamin-dependent protein-trafficking pathway *J Virol* **79** 547-553
- Suzuki Y and Gojobori T (1997) The origin and evolution of Ebola and Marburg viruses *Mol Biol Evol* **14** 800-806
- Swanepoel R, Leman P A, Burt F J, Zachariades N A, Braack L E, Ksiazek T G, Rollin P E, Zaki S R and Peters C J (1996) Experimental inoculation of plants and animals with Ebola virus *Emerg Infect Dis* **2** 321-325
- Takada A, Feldmann H, Ksiazek T G and Kawaoka Y (2003) Antibody-dependent enhancement of Ebola virus infection *J Virol* **77** 7539-7544
- Tan D X, Korkmaz A, Reiter R J and Manchester L C (2014) Ebola virus disease: potential use of melatonin as a treatment *J Pineal Res* **57** 381-384
- Taylor D J, Ballinger M J, Zhan J J, Hanzly L E and Bruenn J A (2014) Evidence that ebolaviruses and cuevaviruses have been diverging from marburgviruses since the Miocene *Peer J* **2** e556
- Taylor D J, Dittmar K, Ballinger M J and Bruenn J A (2011) Evolutionary maintenance of filovirus-like genes in bat genomes *BMC Evol Biol* **11** 336
- Thi EP, Lee ACH, Geisbert JB, Ursic-Bedoya R, Agans KN, Robbins N *et al.* (2016) rescue of non-human primates from advanced *Sudan Ebola virus* infection with lipid encapsulated siRNA *Nature Microbio* **16142**
- Thi E P, Mire C E, Lee A C, Geisbert J B, Zhou J Z, Agans K N, Snead N M, Deer D J, Barnard T R *et al.* (2015) Lipid nanoparticle siRNA treatment of Ebola-virus-Makona-infected nonhuman primates *Nature* doi: 10.1038/nature14442
- Tapia M D, Sow S O, Lyke K E, Haidara F C, Diallo F, Doumbia M *et al.* (2016) Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial *Lancet Infect Dis* **16** 31-42
- Towner J S, Rollin P E, Bausch D G, Sanchez A, Crary S M, Vincent M, Lee W F *et al.* (2004) Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome *J Virol* **78** 4330-4341
- Urbanovicz R A, McClure C P, Sakuntabhai A, Sall A A, Kobinger G, Muller M A *et al.* (2016) Human adaptation of Ebola virus during the west African outbreak *Cell* **167** 1079-1087
- van Hook A M (2014) How Ebola shuts down antiviral signalling *Sci Signal* **7** ee216
- Varkey J B, Shantha J G, Crozier I, Kraft C S, Lyon M, Mehta A K, Kumar G, Smith J R, Kainulainen M H, Whitmer S *et al.* (2015) Persistence of Ebola virus in ocular fluid during convalescence *New Engl J Med* doi: 10.1056/NEJMoa1500306
- Veljkovic V, Loiseau P M, Figadere B *et al.* (2015) Virtual screen for repurposing approved and experimental drugs for candidate inhibitors of EBOLA virus infection *F1000Res* **4** 34 10.12688/f1000research.6110.1
- Villinger F, Rollin P E, Brar S S, Chikkala N F, Winter J, Sundstrom J B, Zaki S R, Swanepoel R, Ansari A A and Peters C J (1999) Markedly elevated levels of interferon (IFN)-gamma, IFN-alpha, interleukin (IL)-2, IL-10, and tumor necrosis factor-alpha associated with fatal Ebola virus infection *J Infect Dis* **179** S188-191
- Volchkov V E, Becker S, Volchkova V A, Ternovoj V A, Kotov A N, Netesov S V and Klenk H D (1995) GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases *Virology* **214** 421-430

- Volchkov V E, Feldmann H, Volchkova V A and Klenk H D (1998) Processing of the Ebola virus glycoprotein by the proprotein convertase furin *Proc Natl Acad Sci USA* **95** 5762-5767
- Volchkova V A, Klenk H D and Volchkov V E (1999) Delta-peptide is the carboxy-terminal cleavage fragment of the nonstructural small glycoprotein sGP of Ebola virus *Virology* **265** 164-171
- Wahl-Jensen V M, Afanasieva T A, Seebach J, Stroher U, Feldmann H and Schnittler H J (2005) Effects of Ebola virus glycoproteins on endothelial cell activation and barrier function *J Virol* **79** 10442-10450
- Walley K R (2011) Use of central venous oxygen saturation to guide therapy *Am J Respir Crit Care Med* **184** 514-520
- Walsh M G and Haseeb M A (2015) The landscape configuration of zoonotic transmission of Ebola virus disease in West and Central Africa: interaction between population density and vegetation cover *Peer J* **3** e735
- Walsh K B, Tejjaro J R, Wilker P R, Jatzek A, Fremgen D M, Das S C et al. (2011) Suppression of cytokine storm with a sphingosine analog provides protection against pathogenic influenza virus *Proc Natl Acad Sci U S A* **108** 12018-12023
- Warren T K, Warfield K L, Wells J, Enterlein S, Smith M, Ruthel G et al. (2010) Antiviral activity of a small-molecule inhibitor of filovirus infection *Antimicrob Agents Chemother* **54** 2152-2159
- Warren T K, Wells J, Panchal R G, Stuthman K S, Garza N L, Van Tongeren S A et al., (2014) Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430 *Nature* **508** 402-405
- Warren T, Jordan R, Lo M, Soloveva V, Ray A, Bannister V et al. (2015a) Nucleotide Prodrug GS-5734 is a broad-spectrum Filovirus inhibitor that provides complete therapeutic protection against the development of Ebola virus disease (EVD) in infected non-human primates <https://idsa.confex.com/idsa/2015/webprogram/Paper54208.html>
- Warren T K, Jordan R, Lo M K, Ray A S, Mackman R L, Soloveva V, Siegel D et al. (2016) Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys *Nature* **531** 381-385
- Warren T K, Whitehouse C A, Wells J, Welch L, Heald A E, Charleston J S, Sazani P, Reid S P, Iversen P L, Bavari S (2015b) A single phosphorodiamidate morpholino oligomer targeting VP24 protects rhesus monkeys against lethal Ebola virus infection *MBio* **6** e02344-14
- Watanabe S, Noda T and Kawaoka Y (2006) Functional mapping of the nucleoprotein of Ebola virus *J Virol* **80** 3743-3751
- Watanabe S, Noda T, Halfmann P, Jasenosky L and Kawaoka Y (2007) Ebola Virus (EBOV) VP24 inhibits transcription and replication of the EBOV genome *J Infect Dis* **196** S284-S290
- Wauquier N, Becquart P, Gasquet C and Leroy E M (2009) Immunoglobulin G in Ebola Outbreak Survivors, Gabon *Emerg Infect Dis* **15** 1136-1137
- Wauquier N, Becquart P, Padilla C, Baize S and Leroy E M (2010) Human fatal zaire ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis *PLoS Negl Trop Dis* **4** e837
- Weissenhorn W, Calder L J, Wharton S A, Skehel J J and Wiley D C (1998) The central structural feature of the membrane fusion protein subunit from the Ebola virus glycoprotein is a long triple-stranded coiled coil *Proc Natl Acad Sci USA* **95** 6032-6036
- Weingartl H M, Nfon C and Kobinger G (2013) Review of Ebola virus infections in domestic animals *Dev Biol* **135** 211-218
- WHO: <http://www.uptodate.com/contents/diagnosis-and-treatment-of-ebola-marburg-virus-disease?source=outline&link&view=text&anchor=H42212845#H422128455>.
- WHO, 2014, <http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/>
- WHO, 2014, <http://www.cdc.gov/vhf/ebola/outbreaks/drc/2014-august.html>
- Williams B R (1999) PKR; a sentinel kinase for cellular stress *Oncogene* **18** 6112-6120
- Wolf T, Kann G, Becker S, Stephan C, Brodt H R, de Leuw P, Grünewald T, Vogl T, Kempf V A, Keppler O T and Zacharowski K (2014) Severe Ebola virus disease with vascular leakage and multiorgan failure: treatment of a patient in intensive care *Lancet* **6736** 62384-62389
- Wong G, Qiu X, Richardson J S, Cutts T, Collignon B, Gren J, Aviles J, Embury-Hyatt C and Kobinger G P (2015) Ebola virus transmission in Guinea pigs *J Virol* **89** 1314-1323
- Wu W and Liu S (2014) Research progress of prevention and treatment of Ebola virus infection *Nan Fang Yi Ke Da Xue Xue Bao* **34** 1519-1522
- Wylie T N, Wylie K M, Herter B N and Storch G A (2015) Enhanced virome sequencing through solution-based capture enrichment *Genome Res* pii: gr.191049.115
- Xu W, Edwards M R, Borek D M, Feagins A R, Mittal A, Alinger J B, Berry K N et al. (2014) Ebola virus VP24 targets a unique NLS binding site on karyopherin alpha 5 to selectively compete with nuclear import of phosphorylated STAT1 *Cell Host Microbe* **16** 187-200

- Yaddanapudi K, Palacios G, Towner J S, Chen I, Sariol C A, Nichol S T and Lipkin W I (2006) Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses *FASEB J* **20** 2519-2530
- Yen C W, de Puig H, Tam J O, Gomez-Marquez J, Bosch I, Hamad-Schifferli K and Gehrke L (2015) Multicolored silver nanoparticles for multiplexed disease diagnostics: distinguishing dengue, yellow fever, and Ebola viruses *Lab Chip* DOI 10.1039/C5LC00055F
- Yoneyama M and Fujita T (2009) RNA recognition and signal transduction by RIG-I-like receptors *Immunol Rev* **227** 54-65
- Yuan S (2015) Possible FDA-approved drugs to treat Ebola virus infection *Infect Dis Poverty* **4** 23
- Yunos N M, Bellomo R, Hegarty C, Story D, Ho L and Bailey M (2012) Association between a chloride-liberal vs chloride-restrictive intravenous fluid administration strategy and kidney injury in critically ill adults *JAMA* **308** 1566-1572
- Zaki S R and Goldsmith C S (1999) Pathologic features of filovirus infections in humans *Curr Top Microbiol Immunol* **235** 97-116.