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Review

Biochemical and virological aspects beyond Ebola virus - Prospects for medications and immunization

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Ebola virus disease (EVD) has a high fatality rate; currently lacks a treatment or vaccine with proven safety. In response, the World Health Organization has declared the Ebola outbreak in West Africa to be a Public Health Emergency of International Concern. However, Ebola is only transmitted by patients who already present symptoms of the disease, and infection only occurs upon direct contact with the blood or body fluids of an Ebola patient. Consequently, transmission of the outbreak can be contained through careful monitoring for fever among persons who have visited, or come into contact with persons from, the site of the outbreak. Thus, patients suspected of presenting symptoms characteristic of Ebola should be quarantined. Despite ongoing efforts directed at experimental treatments and vaccine development, current medical management of EVD is largely limited to supportive therapy, thus making early case identification and immediate implementation of appropriate control measures critical. Optimization of EVD management together with rapid diagnosis, greatly improve the clinical outcome. Recent advances in diagnostic procedures and new therapies of patients with several drugs in the initial phase of treatment could further improve the prognosis of EVD cases. This review summarizes the biochemical and virological characters of Ebola virus and highlights the challenges for development of new effective antiviral drugs and vaccine for prompt control and prevention of EVD outbreaks.

Keywords: Ebola virus disease (EVD), *FILOVIRIDAE*, Marburg virus disease, Outbreaks, ZMapp.

BACKGROUND

Ebola virus belongs to the family *FILOVIRIDAE*; single-stranded non-segmented negative-sense RNA viruses, which shares certain similarities with rhabdoviruses as well as paramyxoviruses regarding genome organization and replication mechanisms. To date, five identified subtypes of the Ebola virus have been identified. Four subtypes have caused infection in humans: Ebola-Zaire, -Sudan, -Bundibugyo, and -Tai Forest. The fifth, Ebola-Reston, has caused infection in nonhuman primates, but not in humans to date. Mortality rates for the Ebola virus disease (EVD)

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range from 34% of the Bundibugyo subtype to 90% of the Zaire subtype, with death usually occurring as a result of shock rather than blood loss (Basler & Amarasinghe, 2009; Wamala *ET AL*., 2010). Although infections only occur frequently in Central Africa, the virus has the potential to spread globally and is classified as a category (A) pathogen that could be misused as a bioterrorism agent (Marzi & Feldmann, 2014).

Outbreaks of the mysterious disease

This mysterious disease was first described in two separate 1976 outbreaks: first in southern Sudan and subsequently in northern Zaire, now Democratic Republic of the Congo. A causative agent was isolated from patients in both epidemics and named Ebola virus after a small river in northwestern Zaire. Only years later did researchers recognize that the plagues were caused by two distinct species of Ebola virus, Sudan Ebola virus and Zaire Ebola virus. The third African species, Cote d'Ivoire Ebola virus was isolated in 1994 from an infected ethnologist who had done a necropsy on a chimpanzee from the Tai Forest. Only in 2007 was a fourth African species; Bundibugyo Ebola virus isolated (Feldmann *ET AL*., 2011).

Since its discovery in 1976 there have been 17 outbreaks of Ebola hemorrhagic fever in Africa. There have been a total of 1860 reported cases of EHF, resulting in 1296 deaths. Undoubtedly, Ebola epidemic in 2014 is the biggest epidemic of this virus, so far, since multiple countries in the West-Africa have been feigned. On August 8, 2014, WHO declared the present West Africa Ebola outbreak as a public health emergency of international concern. Consequently, public health partnerships between the involved countries are expected to be expanded, and the national response systems will be in effect. Beginning in Guinea in December 2013, the present outbreak spread to Sierra Leone and Liberia, and is now the largest outbreak in history. By November 16, 2014, there were 15145 cases (suspected and confirmed diagnoses) and 5420 deaths, representing a 38% fatality rate. By country, Liberia experienced 7069 cases and 2964 deaths, Sierra Leone 6073 cases and 1250 deaths and Guinea 1971 cases and 1192 deaths (WHO: Disease outbreak news, 2014; WHO: Ebola virus disease. Fact sheet N°103, 2014).

Hypothesis of Ebola transmission

Because the natural reservoir of Ebola virus has not yet been identified, the way in which the virus first appears in a human at the start of an outbreak is unknown. However, researchers believe that the first patient becomes infected through contact with an infected animal, such as a fruit bat or nonhuman primate (CDC-Ebola factsheet, 2014).

In Africa, fruit bats of the family Pteropodidae are considered natural hosts of filoviruses – the viruses that cause Marburg and Ebola viruses. Fruit bats belonging to the genus Rousettus are considered potential hosts of the Marburg virus, and bats belonging to the genera Hypsignathus, Epomops, and Myonycteris are considered possible hosts of the Ebola virus. However, Ebola and Marburg have also been found in other bat species. The geographic distribution of Ebola and Marburg viruses probably corresponds to that of fruit bats of the family Pteropodidae (figure 1). Consequently, Ebola and Marburg viruses are considered endemic throughout Sub-Saharan Africa (WHO -Ebola and Marburg virus disease epidemics: preparedness, alert, control, and evaluation, 2014).

Although there are currently no clear indicators regarding the source of Ebola virus, fruit bats of the Pteropodidae family are considered the natural host of the virus, which is also thought to transmit through wild primate animals (monkeys, gorillas, chimpanzees and forest antelopes). Then, Ebola virus spreads through human-to-human transmission via direct contact with the blood, secretions, organs or other bodily fluids of infected people (the most infectious body fluids are blood, feces, and vomit), and with surfaces and materials (e.g. bedding, clothing) contaminated with these fluids.. Ebola virus can also be spread through direct contact with skin of a patient, or though contact with contaminated surfaces and objects (Hayden, 2014). The incubation period for Ebola virus disease ranges from two to 21 days and is characterized by fever, headache, myalgias and gastrointestinal symptoms. Multisystem involvement with hypotension and respiratory, kidney and liver failure may ensue, as well as internal and external bleeding (Paessler, 2013).

Person-to-person transmission of Ebola and Marburg virus occurs through direct contact with the blood, secretions, organs, or other body fluids of infected persons, putting health-care workers and the community at risk. Burial ceremonies in which relatives and friends have direct contact with the body of the deceased person also play a significant role in the transmission of the virus. Health-care workers have been infected while treating Ebola and Marburg patients, through close contact without correct infection control precautions and inadequate barrier nursing procedures. To date, approximately 10% of Ebola or Marburg victims have been health-care workers (WHO: Ebola and Marburg virus disease epidemics: preparedness, alert, control, and evaluation, 2014).

Virion structure

The family name; *FILOVIRIDAE* was derived from the Latin word filum, which alludes to the thread-like appearance of the virions when viewed under an electron microscope (figure 2). The 18.9-kb RNA genome of Ebola virus is non-

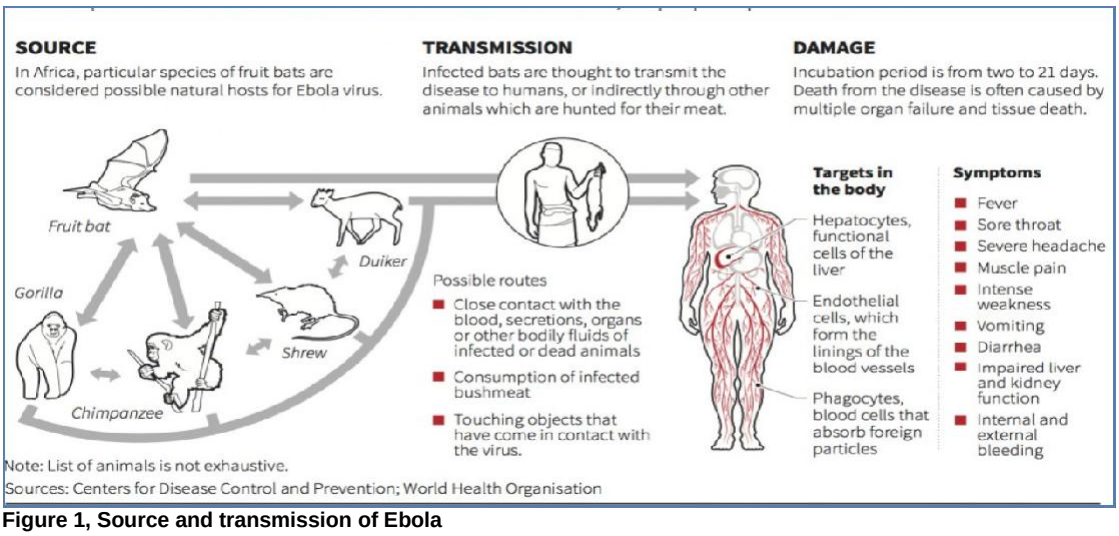

Figure 1, Source and transmission of Ebola

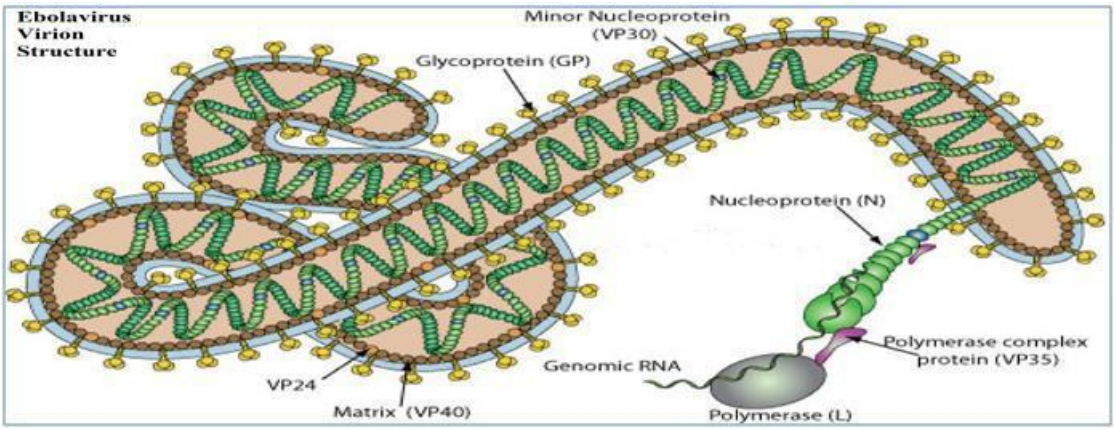

Figure 2. Ebola viron structure (adabted from: Klenk & Feldmann, 2004).

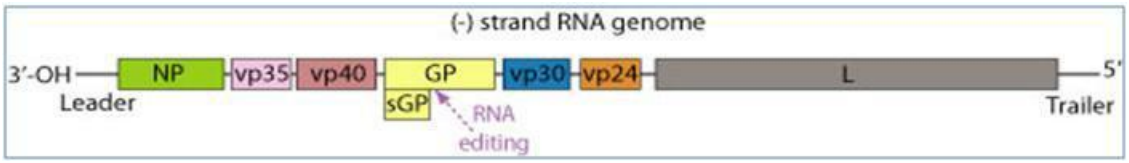

Figure 3. Ebolavirus genome (adapted from Cárdenas *et al*., 2006)

infectious and encodes seven structural proteins and one non-structural protein. Mature Ebola virus particles form long filamentous rods with a uniform diameter of 80 nm and a mean length of1250 nm. Virus particles possess a central core; the ribonucleoprotein complex, that consists of NP, VP35, VP30, L and the viral RNA. This RNP complex is surrounded by a lipid envelope, with which the remaining proteins GP1, and GP2, VP40 and VP24 are associated; these three proteins function as surface glycoprotein, major matrix protein and minor matrix protein, respectively. The Ebola viral proteins play an important role in determining the virulence of Ebola virus sup-types as well as the immune response they elicit in the host cells (Pourrut *ET AL*., 2005).

Filoviruses have been divided into two genera: Ebola-like viruses with species Zaire, Sudan, Reston, Cote d'Ivoire and Bundibugyo; and Marburg-like viruses with the single species Marburg. All of these are responsible for hemorrhagic fevers in primates that are characterized by often fatal bleeding and coagulation abnormalities (Klenk & Feldmann, 2004).

Biochemical Facts

The Ebola virus genome is 19 kb long, with seven open reading frames encoding structural proteins, including the virion envelope glycoprotein (GP), nucleoprotein (NP), and

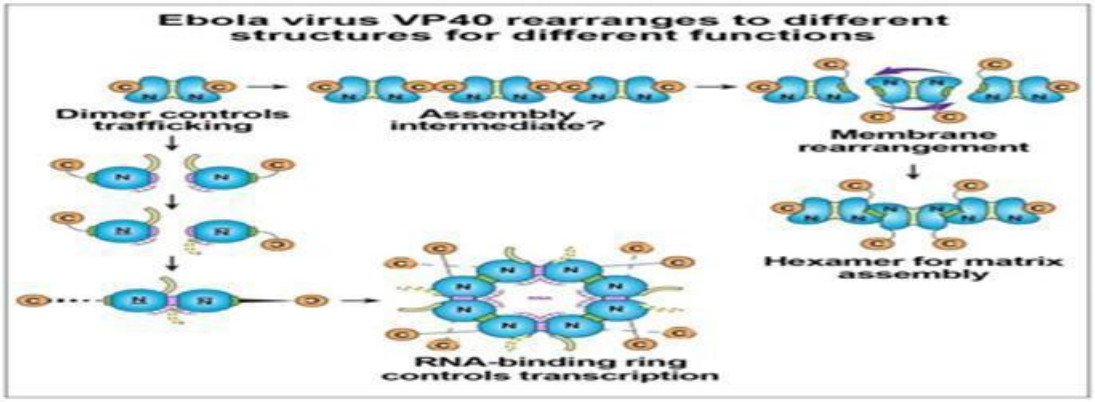

Figure 4. VP40 rearrangement (adapted from Bornholdt *et al.*, 2013)

matrix proteins VP24 and VP40; nonstructural proteins, including VP30 and VP35; and the viral polymerase. Unlike that of Marburg virus, the GP open reading frame of Ebola virus gives rise to two gene products, a soluble 60- to 70-kDa protein (sGP) and a full-length 150- to 170-kDa protein (GP) that inserts into the viral membrane, through transcriptional editing (Sullivan et al, 2003). The genome of each virion is around 19kb in length, and codes for seven structural and one non-structural proteins. The gene order is as follows: 3′ – leader – NP – VP35 – VP40 – GP/sGP – VP30 – VP24 – L – trailer – 5′ (figure 3). The leader and trailer regions are not transcribed, but carry important signals that control transcription, replication and packaging of the genome into new virions (Crary *ET AL*., 2003).

Ebola actually encodes two forms of its glycoprotein gene. The small, non-structural, dimeric soluble form (sGP) is transcribed directly from the viral mRNA and its function remains mostly unknown (Simmons et al., 2002). This protein is not found in virus particles, but is instead secreted from infected cells into the blood (Volchkov et al., 1995). A second glycoprotein results from transcriptional editing of the glycoprotein origin of replication and encodes a trimeric, membrane-bound form. This envelope GP spike is expressed at the cell surface, and is incorporated into the virion to drive viral attachment and membrane fusion. It has also been shown as the crucial factor for Ebola virus pathogenicity (Yonezawa et al., 2005). This protein assembles as a trimer of heterodimers on the viral envelope, and ultimately undergoes an irreversible conformation change to merge the two membranes (Lee et al., 2008). VP40 rearranges into different structures (figure 4), each with a distinct function required for the ebolavirus life cycle. A butterfly-shaped VP40 dimer traffics to the cellular membrane. Once there, electrostatic interactions trigger rearrangement of the polypeptide into a linear hexamer. These hexamers construct a multilayered, filamentous matrix structure that is critical for budding and resembles tomograms of authentic virions (Bornholdt *ET AL*., 2013).

The roles of Ebola virus (EBOV) VP24 in nucleocapsid (NC) formation and the effect of VP24 on transcription and replication of the viral genome during NC formation remain unknown. Watanabe *ET AL*. (2007) examined the effect of VP24 on the expression of a reporter gene (luciferase), viral RNA, and messenger RNA from the EBOV minigenome. VP24 inhibited the expression of luciferase and both RNAs in a dose-dependent manner, suggesting that VP24 inhibits transcription and replication of the EBOV genome (Watanabe *ET AL.*, 2007).

The VP35 protein is a double-stranded RNA (dsRNA) binding protein that inhibits RIG-I signaling and interferon (IFN)-α/β responses by both dsRNA-binding dependent and independent mechanisms. Therefore, VP35 is a general antagonist of dendritic cell (DC) responses to RLR activation. However, Toll-like receptor (TLR) agonists can circumvent many of the inhibitory effects of VP35. This suggests strategies to counteract VP35 immune evasion functions (Yen *ET AL*., 2014).

To enter cells, Ebola virus must bind to target cells and internalize into endocytic vesicles (**Schornberg** *ET AL.*, 2006). Within the endosome, low-pH-dependent proteolysis of the viral surface GP (GP1) is required for GP2-dependent fusion of the virus with cellular membranes (Chandran *ET AL.*, 2005). Infection in humans occurs when the virus binds to macrophages and dendritic cells expressing the TIM-1 receptor. The genome is released into the cytoplasm, translated, and replicated by the RNA-dependent RNA polymerase brought into the cell by the virus. Virions are assembled at the plasma membrane and released by budding to spread throughout the host (Feldman & Klenk, 1996).

Xu *ET AL.* (2014) revealed that during antiviral defense, interferon (IFN) signaling triggers nuclear transport of tyrosine-phosphorylated STAT1 (PY-STAT1), which occurs via a subset of karyopherin alpha (KPNA) nuclear transporters. Many viruses, including Ebola virus, actively antagonize STAT1 signaling to counteract the antiviral effects of IFN. Ebola virus VP24 protein (eVP24) binds

Table 1. Laboratory tests used in diagnosis include

Timeline of Infection	Diagnostic tests available
Within a few days after symptoms begin	- Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing. - IgM ELISA. - Polymerase chain reaction (PCR). - Virus isolation
Later in disease course or after recovery	- IgM and IgG antibodies.
Retrospectively in deceased patients	- Immunohistochemistry testing. - PCR. - Virus isolation.

(CDC-Ebola factsheet, 2014 - http://www.cdc.gov/vhf/ebola/pdf/ebola-factsheet.pdf).

KPNA to inhibit PY-STAT1 nuclear transport and render cells refractory to IFNs. They described the structure of human KPNA5 C terminus in complex with eVP24. In the complex, eVP24 recognizes a unique nonclassical nuclear localization signal (NLS) binding site on KPNA5 that is necessary for efficient PY-STAT1 nuclear transport. eVP24 binds KPNA5 with very high affinity to effectively compete with and inhibit PY-STAT1 nuclear transport. In contrast, eVP24 binding does not affect the transport of classical NLS cargo. Thus, eVP24 counters cell-intrinsic innate immunity by selectively targeting PY-STAT1 nuclear import while leaving the transport of other cargo that may be required for viral replication unaffected.

Diagnosis

Diagnosing Ebola in an individual who has been infected for only a few days is difficult because the early symptoms, such as fever, are nonspecific to Ebola virus infection and are seen often in patients with more common diseases, such as malaria and typhoid fever. However, if a person has the early symptoms of Ebola and there is reason to believe that Ebola should be considered, the patient should be isolated and public health professionals notified. Samples from the patient can then be collected and tested to confirm infection.

Ebola virus is detected in blood only after onset of symptoms, most notably fever, which accompany the rise in circulating virus within the patient's body. It may take up to three days after symptoms start for the virus to reach delectable levels (table 1).

Basic principles for Ebola virus disease (EVD)-case management:

The basic principles for EVD-case management are early recognition and isolation of cases, use of personal protective equipment (PPE), and the provision of supportive medical care to reduce mortality. Therefore, the updated WHO guidelines are aimed at a range of clinicians, both specialist and non-specialist to establish a systematic approach to comprehensive clinical management of EVD cases (WHO. Ebola virus disease, 2014; Meyers *ET AL*., 2014).

There are no FDA-approved vaccines or therapeutics available for prevention, post-exposure, or treatment for Ebola virus infection. However, the FDA opened the "fast track" status for Ebola drugs, and an Ebola vaccine developed by GlaxoSmithKline obtained positive data in animal experiments, is currently undergoing Phase I clinical trials. Tekmira's TKM-Ebola also received FDA "verbally confirmed" changes on August 9, 2014, which may allow the company to make the drug available. However, it is unethical to apply unproven drugs to patients, and it also smacks of injustice that only American patients can try Ebola drugs such as ZMapp while the majority of patients in Africa are without trial drugs (Zhang and Wang, 2014).

WHO is taking aggressive actions. On August 11, 2014, WHO convened a consultation to consider and assess the ethical implications for clinical decision making of the potential use of unregistered interventions. The panel reached consensus that in the particular circumstances of the current outbreak of EVD and provided certain conditions are met, it was ethical to offer unproven interventions with as yet unknown efficacy and adverse effects, as the potential treatment or prevention. However, perhaps in the current circumstances with no cure for the virus, using of experimental drugs is the only option and the only hope. The first shipment of the experimental drug ZMapp arrived in Spain on August 11, 2014 (WHO: Ethical considerations for use of unregistered interventions for Ebola virus disease, 2014). It is noteworthy that Xu et al (2014) found that an Ebola viral protein blocks the transport of an interferon-activated protein called STAT1 into the cell nucleus. STAT1 is needed in the nucleus to stimulate defence mechanisms. The results suggest new drug targets in the ongoing fight against the virus (Xu *ET AL*., 2014).

Monoclonal antibody (mAb) cocktails are particularly attractive candidates due to their proven post-exposure efficacy in nonhuman primate models of EBOV infection. Two candidate cocktails, MB-003 and ZMAb, have been extensively evaluated in both in vitro and in vivo studies. Recently, these two therapeutics have been combined into a new cocktail named ZMapp, which showed increased efficacy and has been given compassionately to some human patients. Epitope information and mechanism of action are currently unknown for most of the component mAbs. Murin *ET AL*. (2014) provided single-particle EM reconstructions of every mAb in the ZMapp cocktail, as well as additional antibodies from MB-003 and ZMAb. Their results illuminated key and recurring sites of vulnerability on the EBOV glycoprotein and provided a structural rationale for the efficacy of ZMapp (Murin et al., 2014).

Prospects for immunization

Experimental vaccines and treatments for Ebola are under development, but they have not yet been fully tested for safety or effectiveness. Recovery from Ebola depends on good supportive care and the patient's immune response. People who recover from Ebola infection develop antibodies that last for at least 10 years, possibly longer. It isn't known if people who recover are immune for life or if they can become infected with a different species of Ebola. Some people who have recovered from Ebola have developed long-term complications, such as joint and vision problems (CDC-Ebola factsheet, 2014).

There are promising candidates in clinical trials for prevention of the disease like DNA vaccines or vaccines derived from adenoviruses, vesicular stomatitis Indiana virus, filovirus-like particles, or recombinant adenovirus vector platform (Ad 26 & Ad 35), or adjuvanted virus-like particles. These vaccines could protect nonhuman primates from Ebola and hopefully can be engaged in human (Geisbert et al., 2008; Phoolcharoen et al., 2011).

As of today there is no vaccine or treatment licensed to counteract Ebola virus infections, DNA-subunit and several viral vector approaches, replicating and non-replicating, have been tested as potential vaccine platforms. Their protective efficacy has been evaluated in nonhuman primate models for Ebola virus infections, which closely resemble disease progression in humans. Though these vaccine platforms seem to confer protection through different mechanisms, several of them are efficacious against lethal disease in nonhuman primates attesting that vaccination against Ebola virus infections is feasible (Marzi & Feldmann, 2014). Due to the generally remote locations of filovirus outbreaks, a single-injection vaccine is desirable. Among the prospective vaccines that have shown efficacy in nonhuman primate models of filoviral hemorrhagic fever, two candidates, one based on a replication-defective adenovirus serotype 5 and the other on a recombinant VSV (rVSV), were shown to provide complete protection to nonhuman primates when administered as a single injection. The rVSV-based vaccine has also shown utility when administered for post-exposure prophylaxis against filovirus infections. A VSV-based Ebola vaccine was recently used to manage a potential laboratory exposure (Geisbert et al., 2010).

With the Ebola epidemic in West Africa continuing to grow, WHO convened an urgent meeting on September 29 and 30 to assess the efforts under way to evaluate and produce safe and effective Ebola vaccines as soon as possible (WHO: Experimental Ebola vaccines, 2014). The 70 scientists, public health officials, and representatives from industry and regulatory bodies who gathered in Geneva discussed two vaccine candidates at length — cAd3-EBOV (cAd3), from GlaxoSmithKline (GSK) and the U.S. National Institute of Allergy and Infectious Diseases (NIAID), and rVSV∆G-EBOV-GP (rVSV), from NewLink Genetics and the Public Health Agency of Canada (Kanapathipillai *ET AL*., 2014)**.**

Phase 1 studies of cAd3 have begun in the United States and the United Kingdom, and researchers plan to begin enrollment for trials of rVSV soon. Both vaccine candidates have demonstrated 100% efficacy in studies in nonhuman primates, but how that will translate to human subjects remains unknown. The cAd3 vaccine is being tested in both bivalent (ClinicalTrials.gov number, NCT02231866) and monovalent (NCT02240875) forms; the monovalent form is based on the Zaire strain of Ebola virus, which is the cause of the current West African epidemic, and the bivalent form includes the Sudan strain of the virus as well**.** The first phase 1 trial of the rVSV vaccine is slated to begin soon in the United States. Ideally, the immunogenicity outcomes in this trial will be compared with those obtained with the GSK–NIAID vaccine. The government of Canada

has donated 800 vials of rVSV to the WHO, and discussions about expanding phase 1 trials to European and sub-Saharan African sites are at an advanced stage (Kanapathipillai *ET AL*., 2014)**.**

Participants in the Geneva meeting stressed that phase 1 trials should be expedited and their results shared broadly in order to facilitate rapid progression to phase 2. If the results in phase 1 are favorable, the consensus was that phase 2a studies should be conducted in Africa but outside the current Ebola outbreak zone and should proceed in parallel with phase 2b studies conducted in exposed populations. This approach will provide robust efficacy and safety data as quickly as possible. Results from phase 2a trials in unexposed populations would inform the use of these vaccines in expanded populations, including children and people who are HIV-positive. The phase 2b trials in exposed populations would enroll people who are at the highest risk for Ebola virus disease, including frontline workers at Ebola treatment facilities (Kanapathipillai *ET AL*., 2014)**.**

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