Ebola: Working Toward Treatments and Vaccines
Claire Sykes and Miriam Reisman

The deadly and highly infectious Ebola virus was discovered in 1976 in Yambuku in the Democratic Republic of Congo, 60 miles from the river after which it was named.1 But until the 2014 Ebola outbreak in the equatorial West African countries of Liberia, Guinea, and Sierra Leone, it had never before spread so rapidly and so widely.2 Here, from remote villages to dense urban areas, the total number of Ebola cases has exceeded 27,600, with more than 11,200 deaths—ranking this epidemic as the largest on record for the number of people and the geographic reach.3 Because of its vicious effects, the Ebola virus is classified by the U.S. Centers for Disease Control and Prevention (CDC) as a potential “category A” bioterrorism agent (the highest priority).4

First reported in March 2014, the epidemic in West Africa has subsided significantly. In June, 104 new cases were confirmed, including the first two cases in Liberia since March.5 But the development of an effective, emergency antiviral countermeasure for Ebola remains a prime concern for the CDC and the U.S. Department of Defense (DOD) as the virus continues to emerge in new locations. Despite decades of considerable research efforts, no vaccines or therapeutics are currently licensed for the prevention or treatment of infection by the Ebola virus.

How the Ebola Virus Works

Ebola is caused by infection with a virus of the family Filoviridae, genus Ebolavirus. There are five strains of the Ebola virus, each named for the region where it was originally identified (Figure 1). Four of these strains are known to cause disease in humans: Ebola virus (Zaire ebolavirus), which caused the 2014 outbreak; Sudan virus (Sudan ebolavirus); Tai Forest virus (Tai Forest ebolavirus, formerly Côte d’Ivoire ebolavirus); and Bundibugyo virus (Bundibugyo ebolavirus). The fifth, Reston virus (Reston ebolavirus), has caused disease in nonhuman primates but not in humans.2

Ebola is an elongated, single-stranded virus (Figure 2) that enters the host primarily through mucous membranes or scraped skin and easily spreads to others by infected bodily fluids, primarily blood, saliva, emesis, or stool; it does not appear to be transmitted through the air. Four to 10 days following the incubation period, a person infected with Ebola experiences fever, chills, myalgia, and a general malaise—symptoms that might be confused with the flu or any number of other complaints. The virus is not spread until the patient becomes symptomatic. Within a maximum of 21 days, the patient develops vomiting, abdominal pain, and diarrhea, followed by hemorrhaging, coagulopathy, and cytopenia, among other symptoms—indicative of a severe and perhaps fatal infection.

Because the natural host of the Ebola virus has not yet been identified, it is not known how an outbreak occurs in humans. However, based on evidence and the nature of similar viruses, researchers believe that the virus is animal-borne, with fruit bats being the most likely reservoir. Four of the five virus strains occur in an animal host native to Africa.2

Front-Runner Drugs for Ebola Treatment

Although no treatments approved by the U.S. Food and Drug Administration (FDA) are currently available for Ebola, a number of experimental products have shown promising signs of effectiveness against the virus. Approximately 30 Ebola drug candidates are undergoing investigation. As of early June 2015, five of these drugs are in clinical development:

TKM-Ebola

TKM-Ebola, an RNA interference (RNAi) therapeutic, is being developed by Tekmira Pharmaceuticals of Vancouver, British Columbia, in collaboration with the DOD. In March 2015, a new phase 2 trial of TKM-Ebola began in Sierra Leone. Designed specifically to target the strain of the Ebola virus responsible for the outbreak in West Africa, the therapeutic

Figure 1 Ebola Virus Outbreaks by Species and Size, 1976–20156

Figure 2 The Ebola Virus7

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works by blocking certain genes of the virus, thereby reducing viral replication. An earlier version of the TKM-Ebola drug (targeting a different strain) has been tested in healthy human volunteers. The new version will be evaluated in patients with known Ebola-virus infection to see if it can improve survival. Results of the study are expected in the second half of 2015.8

ZMapp
ZMapp, the widely reported antibody cocktail that was given under emergency authorization to several infected aid workers at the height of the epidemic, began clinical testing for safety and efficacy in February 2015.9 The trial, a joint effort by the Liberian government and the U.S. National Institute of Allergy and Infectious Diseases (NIAID), is being conducted in Liberia

AVI-7537: An Inside Look at an Ebola Drug in Development

From 2004 to 2012, Patrick L. Iversen, PhD, Distinguished Scientist at Sarepta Therapeutics and Professor in the Environmental Molecular Toxicology Department at Oregon State University in Corvallis, Oregon, conducted a series of trials funded by the U.S. Department of Defense to discover a test article for the Ebola virus, as well as the related Filoviridae virus Marburg.26,27

All of Dr. Iversen’s mouse, guinea pig, and non-human primate lethal-challenge models for his study were performed by investigative colleagues at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). The Biosafety Level (BSL) 3 and Level 4 military research facility in Frederick, Maryland, develops vaccines, drugs, diagnostics, and medical information, playing an essential role in civilian biodefense research. And it’s a very expensive place to do research. “One primate study could easily cost $2 million. For mice it could be $50,000 and guinea pigs $100,000. It’s not the studies themselves that increase the cost, but the paperwork to the FDA due to hiring for quality assurance and control,” says Sina Bavari, Chief Scientific Officer for USAMRIID.

“In order to work here with the Ebola virus, and any lethal substance, you have to do so with someone who has had USAMRIID certification for at least one year,” says Dr. Iversen, who has never been inside the facility’s BSL-4 lab, where his trials for the AVI-7537 study were conducted. “The lab demands highly trained people. The responsibility is extraordinary, as much as for working with nuclear weapons. Just one small test tube of an Ebola blood sample can contain 10 billion viral particles. That’s enough to kill everyone on the planet, and then some, since the earth’s population is only six billion. If an infected monkey bit or scratched anyone through their positive-pressure suit in the lab, the highly trained researcher might die from the infection. That’s why, in case of a lab accident, which is highly unlikely, it’s critical to have an antiviral like AVI-7537 that’s readily available, so the person could be treated rapidly.”

Dr. Iversen and colleagues at AVI BioPharma developed a synthetic antisense phosphorodiamidate morpholino oligomer (PMO) designated as AVI-6002 (composed of AVI-7537 and AVI-7539). The discovery process first identified optimal transcript binding sites in vitro for RNA-targeted PMO therapeutics. Using adapted viral isolates, Dr. Iversen then screened for effective gene targets in mice and guinea pigs, which resulted in two agents combined to target separate genes (AVI-6002). Next, the team of investigators tested chemical changes to the PMOs on these animals with three different types of PMOs. After starting with PMOs, Dr. Iversen moved on to cell-penetrating peptide-conjugated PMMOs and finished with PMOplus. PMOplus is a Sarepta proprietary, adaptable platform chemistry that contains a selected number of positively charged linkages in the form of piperazine residues at specific locations along the PMO backbone. The PMOplus platform comes with advantages for antisense complexes, improving their stability (making them resistant to degradation, compared to conventional antisense methods), function, bioavailability, and binding kinetics.

PMOplus is stable because it is a morpholino analog that has phosphorodiamidate linkages; this basic structure is resistant to degradation by RNA and DNA enzymes. In addition, the positive charge from the piperazine ring is believed to improve the binding of the PMOplus to the target messenger RNA, which may enhance activity against Ebola, according to Alison Heald, Clinical Assistant Professor at the University of Washington in Seattle and lead researcher of a follow-up phase 1 study evaluating AVI-7537 in 30 healthy men and women.10 “The PMOplus platform has enormous potential because the safety profile is attributed to the PMO chemistry, not the antisense sequence,” she says.

By study’s end, Dr. Iversen determined that the PMOplus AVI-7537 was the only test article needed to successfully suppress the Ebola virus in infected non-human primates. “We landed on AVI-7537 by looking for compounds that would be active against a particular gene,” he says. “Filoviruses have seven genes of the Ebola virus—L, VP24, VP30, GP, VP40, VP35, and NP. We looked at their various controlling elements and determined there were three genes that gave us nice responses in the Ebola virus—VP35, VP24, and L. We ruled out two genes with the mice and guinea pig trials, and with the nonhuman-primate trials confirmed VP24 as the key viral gene, with AVI-7537 as the antiviral with the most efficacy and safety.”

To arrive at this single-agent conclusion, Dr. Iversen had asked whether both AVI-7537 and AVI-7539 (the components of AVI-6002) were necessary for the agent’s efficacy, as well as for preventing Ebola-virus resistance. Targeting VP24, he found that AVI-7537 alone offered greater survival than when it was combined with AVI-7539 and that AVI-7539 by itself showed no survival benefit. The data told him that the main contributor to efficacy (as observed with
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and the U.S. among adults and children with a confirmed diagnosis of Ebola-virus infection.

Developed by Mapp Biopharmaceutical Inc. of San Diego, California, ZMapp is made up of three different monoclonal antibodies that work to prevent the spread of the Ebola-virus disease within the body.

AVI-7537

AVI-7537 demonstrated significant efficacy in five independent studies conducted in Ebola-infected rhesus monkeys. Developed by Sarepta Therapeutics, an RNA-based biopharmaceutical company in Cambridge, Massachusetts, AVI-7537 was also found to be safe and well tolerated at the doses tested in a phase 1a clinical trial.

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The virtue of AVI-7537 is that it can be manufactured on demand, anticipating the Ebola virus’s capacity to mutate readily, and made available in the event of an accident or outbreak. The DOD has been evaluating AVI-7537 as a postexposure prophylaxis for the Ebola virus. Meanwhile, Sarepta Therapeutics has had discussions with the FDA and the European Medicines Agency about its use under an emergency request.

Favipiravir

Favipiravir, also known as T-705 or Avigan, is an RNA polymerase inhibitor undergoing phase 2 efficacy testing for Ebola treatment in Guinea.11 Developed by Tokyo-based Toyama Chemical, favipiravir is unique in that it has already been tested against the influenza virus in adult humans and was well tolerated.12 Preliminary results reported in February 2015 showed favipiravir may be effective against the Ebola virus if given early in the illness.13

BCX4430

BCX4430, developed by BioCryst Pharmaceuticals of Durham, North Carolina, is a novel synthetic adenosine analogue that inhibits infection by distinct filoviruses in human cells. The drug has been demonstrated to protect against the Ebola and Marburg viruses in rodents and monkeys, even when administered up to 48 hours after infection.14 With funding from the U.S. National Institutes of Health (NIH), BCX4430 is undergoing phase 1 safety studies in healthy volunteers.15

Multiple Ebola Vaccines in Development

At a meeting in August 2014, the Emergency Committee of the World Health Organization (WHO) concluded that the Ebola outbreak in West Africa continued to constitute a “public health emergency of international concern.” Since then, the speed with which Ebola vaccines are being developed has been expedited, with clinical trials for several candidates in various phases.

Currently, a phase 2/3 safety and effectiveness study for the experimental vaccine rVSV-ZEBOV is under way in Sierra Leone.16,17 The trial program, known as STRIVE (Sierra Leone Trial to Introduce a Vaccine against Ebola), is set to enroll at least 6,000 health care and other frontline workers. Developed by NewLink Genetics and Merck Vaccines USA in collaboration with the Public Health Agency of Canada, rVSV-ZEBOV has also undergone testing in Guinea, the United States, Canada, and other countries. The STRIVE trial is sponsored by the CDC, Sierra Leone’s Health Ministry, and the University of Sierra Leone’s College of Medicine and Allied Health Sciences. Earlier research showed that the rVSV-ZEBOV vaccine is safe and elicited a robust immune response in all 40 healthy adult volunteers.18

Another Ebola vaccine candidate, ChAd3-ZEBOV, entered phase 2 and 3 efficacy trials in Liberia and Guinea in February 2015 and March 2015, respectively.17,18 Developed by GlaxoSmithKline in collaboration with NIAID, the vaccine uses a chimpanzee adenovirus vector to deliver Ebola-virus genetic material from both the Zaire and Sudan strains of Ebola. Phase 1 safety trials demonstrated that ChAd3-ZEBOV caused no serious side effects and produced an immune response in all 20 healthy volunteers.19

Most recently, an Ebola vaccine that can be inhaled has been found to neutralize the deadly virus in monkeys, according to a study published in July 2015 by researchers at the University of Texas Medical Branch and the NIH. A first of its kind, the aerosolized vaccine will advance to phase 1 clinical trials once approved.21 A number of other Ebola vaccines that are being tested also look promising.17

- Ad26-EOBV and MVA-EOBV, developed by Johnson & Johnson in association with Bavarian Nordic, entered phase 1 testing in January 2015. The two-dose approach is known as heterologous prime-boost and uses different vaccines for the first and second doses.
- Novavax, a U.S. biotech company, is developing a recombinant protein Ebola-vaccine candidate based on the Guinea 2014 Ebola-virus strain and has begun phase 1 human clinical trials in Australia.
- The Russian Federal Ministry of Health is developing a recombinant influenza-candidate Ebola vaccine that is scheduled to start phase 1 trials in the second half of 2015.
- The Venezuela equine encephalitis replicon Ebola vaccine, developed by the U.S. Army Medical Research Institute of Infectious Diseases in Maryland, is in the preclinical stage, with no stated time frame for phase 1 trials.

Information on clinical trials of candidate Ebola vaccines is available at www.ClinicalTrials.gov.

Falling Incidence and Other Research Challenges

The Ebola outbreak is waning but not yet contained. According to the CDC, the epidemic will officially be declared over when 42 days, or two full incubation periods, have passed after the last case is treated.22 As efforts shift from fighting the Ebola epidemic to eliminating the virus and as pharmaceutical companies face mounting pressure to ramp up research and development, a host of logistical, ethical, and financial challenges looms large.

The sharp drop in infections signals a positive turning point in the Ebola crisis, but it has also made it more difficult to enroll patients in clinical trials. In February 2015, the drug company Chimerix, of Durham, North Carolina, announced that it was halting its trial of the antiviral drug brincidofovir at a clinic in Monrovia, Liberia. According to the company’s chief executive, too few patients were enrolled to reach any definitive conclusion.

Vaccine trials are facing the same dilemma: It is hard to determine how well study participants are protected from the Ebola virus if they are not actually exposed to it. In Sierra Leone, where the STRIVE trial for the rVSV-ZEBOV vaccine aims to enroll 6,000 health care workers, the number of newly reported Ebola cases dropped to 21 in early April 2015, prompting the trial to reach any definitive conclusion.23

Falling incidence and other research challenges

Ebola drug trials raise ethical concerns, as well, including whether placebos should be given to a control group and whether experimental treatments that have never been tested in humans should be offered to virus-infected patients. WHO convened a panel in August 2014 to address the ethical implications of the use of unproven interventions for Ebola-virus disease as potential treatment or prevention.25 The panel reached a consensus that in the existing circumstances it is
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ethical to offer these unproven interventions. However, the panel also advised that “certain ethical criteria must guide the provision of such interventions and should include: transparency about all aspects of care; informed consent; freedom of choice; confidentiality; respect for the person; preservation of dignity; and involvement of the community.”

The economics of Ebola drug development pose further challenges to clinical trials, as there is little financial incentive for pharmaceutical companies to invest in treatments for a rare disease that has appeared only sporadically in low-income African countries. In fact, Ebola research has been funded far more by government grants than by drug companies, and federal budget constraints add to the already difficult task of bringing these drugs to market. As of October 2012, the DOD stopped funding Sarepta Therapeutic’s efforts in the development of the Ebola virus, not because of safety or efficacy issues but because of government funding cuts. However, Michael Wong, MD, Sarepta’s Senior Medical Director, said that the company is having conversations with the FDA to work out the next steps for AVI-7537.

In early 2015, there were some initial interest and efforts by the Wellcome Trust, an international charitable foundation in London, to assess the clinical feasibility and evaluation of all investigational agents for Ebola in West Africa. Because an internal infrastructure had to be developed in those countries hit hardest and because on-the-ground medical support was severely affected by the outbreak, international efforts were redirected to shore up those areas first.

“It’s incredibly difficult to design and execute clinical evaluation of new drugs in rural areas of West Africa, because of infrastructure, resources, the location of the treatment facilities, and ethics,” said Patrick Iversen, PhD, of Sarepta Therapeutics. “Some of the smaller villages are very hard to get to, let alone monitor how many people are dying there. I think it’s too big of an endeavor for anyone; no drug company would want to do it. In fact, with the new case rate declining rapidly in West Africa, the ability to enroll in the now-established clinical trial for oral agents has been hindered tremendously. We may not get any answers. Then what? That’s the question. And it troubles me a lot. Ebola may not be killing as many people, so it’s not so much in the news right now. But the virus is a resident on this planet. It’s smoldering and active in the waning endemic, but it will eventually spread to other places. Who knows where or when?”

REFERENCES