Unraveling EBOLA

Scientists are deciphering the virus’s inner workings in an effort to develop effective drugs.

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After Craig Spencer, the American doctor who contracted Ebola while volunteering in Guinea, walked out of a New York City hospital last month, national anxiety over Ebola seemed to dissipate. Days earlier, quarantines had been lifted on those who had interacted with him.

America relaxed, but the epidemic rages on in West Africa. Even as evidence mounts that the outbreak is abating in some regions, flare-ups in other areas make experts reluctant to say the epidemic is under control. To date, more than 15,300 people have contracted the virus in Sierra Leone, Guinea, and Liberia. Of them, nearly 5,500 have died.

Those numbers weigh heavily on researchers who have dedicated their careers to understanding and developing drugs against the Ebola virus. Their labs are far away from the battlefield, but they are still on the front lines. Finding effective treatments could do more than just quell the current outbreak. Critically, it could prevent future ones.

“The pressure is tremendous,” says Travis K. Warren, a scientist at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), who has worked on several of the Ebola treatments that have been tested on American and European patients. USAMRIID, like many other labs with expertise in Ebola, has set aside all other work to determine as quickly as possible which treatments are most promising, to gather more information about how the virus interacts with different drugs, and to assess whether any other untested molecules might also be effective against the virus.

That work is being done at a breathtaking pace. Some programs have gone from the petri dish to tests in monkeys in the span of a month, says Sina Bavari, chief of molecular and translational sciences at USAMRIID. “We’re doing studies on top of studies to just make sure that we can appropriately respond to the outbreak,” Bavari adds.

But scientists have been trying to conquer Ebola for almost 40 years. The question lingers: Why has it been so hard to develop a drug to take down the virus? Researchers point to a deceptively simple system—each Ebola virus particle is little more than a single strand of RNA coated with proteins and encased in two layers of lipids—that does a bang-up job of fooling its host.

“I like to think of the virus as a guided missile,” says Kartik Chandran, a microbiologist at Albert Einstein College of Medicine. “It’s got a payload and a delivery system.”

That RNA payload is surprisingly small. The Ebola virus is endowed with just seven genes that synthesize a mere eight proteins. By contrast, humans have more than 20,000 genes.

But don’t let the simplicity fool you. Each protein made by the Ebola virus is as multifunctional as a Swiss Army knife. And the virus also will beg, borrow, and steal to survive: USAMRIID researchers found that the virus uses nearly 70 proteins from its host. It is so resourceful that just a few particles—possibly even one—can be lethal.

To find starting points for drug development, researchers have spent years trying to unravel the form and function of the proteins the Ebola virus makes as well as where and how they interact with the proteins of its human hosts. They’ve tried a
range of therapeutic approaches—antibodies, small molecules, RNAi-based drugs—to prevent it from replicating.

The drugs attempt to hit the virus at various stages as it settles into its host. Knock out the right protein, block the right landing spot on the host, and perhaps the virus can be stopped in its tracks. Keep it from copying its genetic blueprint, or from building the proteins it needs to multiply, and maybe it will be too crippled to survive. Researchers are trying all of these.

HOME IN ITS HOST

After it finds its way into the body through, for example, a drop of blood, the first thing the Ebola virus needs to do to survive is put down an anchor. That anchor is the Ebola virus glycoprotein, a huge, heavily glycosylated molecule that attaches to the surface of the cell, setting the stage for the virus to sneak inside, unleash its genetic payload, and start replicating.

For years, researchers have sought some way to keep the virus adrift. One strategy now being pursued in the clinic is to use convalescent serum, which comes from the blood of Ebola survivors and provides antibodies that fight the virus. Another is to develop antibodies directed against the glycoprotein. Indeed, ZMapp, the “secret serum” given to Kent Brantly and Nancy Writebol, the first two people to be treated in the U.S. for Ebola, was a mixture of three antibodies that bind to the protein and keep it from anchoring inside a host cell.

Doctors don’t know whether ZMapp, which was developed by the tiny biotech firm Mapp Biopharmaceutical, actually helped Brantly and Writebol overcome the virus, or if their survival was instead due to the intensive care they received at Emory University Hospital. But researchers think an antibody cocktail that targets the Ebola glycoprotein is likely the best hope right now for a treatment that could work after someone is symptomatic.

Amazingly, the antibody cocktail didn’t exist a year ago, according to Erica Ollmann Saphire, a structural biologist at Scripps Research Institute in La Jolla, Calif., whose lab focuses on viruses.

The mixture was the result of painstaking work to understand the structure and function of the Ebola glycoprotein. A breakthrough came in 2008, when Ollmann Saphire’s lab solved the structure of the glycoprotein bound to an antibody from an Ebola survivor. The crystal structure revealed several floppy sugar molecules that form a canopy over the portion of the glycoprotein responsible for binding inside the host cell.

Once the glycoprotein is inside the endosome—a cellular sorting compartment that typically breaks down proteins for recycling—one of the host’s enzymes, called cathepsin, snips off the glycan shield. What’s revealed is the portion of the protein that attaches to the host.

That canopied structure not only provided starting points for drug discovery but also explained why earlier efforts to develop antibodies against the virus failed: Some antibodies target parts of the glycoprotein that are lopped off in the endosome, leaving the critical core of the molecule free to do its business.

Right now, researchers developing therapeutics against the glycoprotein don’t fully understand why the three antibodies in ZMapp seem to work—or even if all three are required.

Because of the elaborate glycan shield and the speed of replication, among other factors, more than one antibody is likely required to do the trick, Ollmann Saphire says. So far, though, the only evidence to support that hypothesis comes from studies of KZ52, an antibody retrieved from the bone marrow of a survivor of a 1995 Ebola outbreak in Zaire. Although KZ52 cured mice with Ebola, it did not save monkeys with the disease.

It turns out that KZ52 blocks most, but not all, of the viral particles, Ollmann Saphire explains. And just a few, or maybe even one, of them can be deadly. “It’s a really tall order to ask one monoclonal antibody to scrub out every viral particle,” she says.

Since the outbreak, Ollmann Saphire’s lab has also been working around the clock to understand where the antibodies in ZMapp bind. Last month, she and fellow Scripps scientist Andrew Ward showed that two of them seem to be going after the same site on the glycoprotein. The researchers now wonder whether the cocktail being used makes the most sense or if it would be better to choose an antibody that blocks a new site on the virus.

Right now there is a clamoring, ‘Open your cupboards.’”

WELCOME TO THE ENDOSOME

Pruning the glycoprotein’s sugary shield reveals what scientists think is another promising avenue for drug discovery. Sitting on that pared-down glycoprotein is a receptor that binds to a membrane protein in the host cell called Niemann-Pick C1. Normally, this protein’s job is to shuttle cholesterol within the cell. Mutations to the gene that makes the protein result in the rare, fatal Niemann-Pick disease.

Ebola needs the Niemann-Pick C1 protein to infect a host cell, Einstein’s Chandran says. Indeed, cells from patients with Niemann-Pick type C1 disease resist Ebola infection, something scientists learned only a few years ago. If scientists can find small-molecule inhibitors that block the viru-
disguised as RNA nucleotide components. The viral polymerase incorrectly identifies the decorated drugs as RNA building blocks and starts incorporating them into the copies of RNA that it's making to replicate the virus. "They’re sort of like poisons that prevent viral genomes from being copied,” Chandran says.

BCX4430, for example, is thought to look to viral RNA polymerases like the purine base adenosine. “It creates a distortion in the new RNA molecule such that only one or two additional bases are able to be added before the polymerase realizes a mistake has been made and terminates the synthesis reaction,” explains USAMRIID’s Warren, who has worked with BCX4430.

The challenge to designing such antivirals is to make them able to trick the viral polymerase without also fooling the host cell’s polymerases. If the host polymerases can’t make the distinction, Warren says, the drug will almost certainly be toxic.

Scientists think that favipiravir works similarly to BCX4430. Warren tells C&EN that 2APR provided evidence that favipiravir, which is made by the biotech firm Chimerix, is currently in Phase III trials to treat both adenovirus and cytomegalovirus. Three of the Ebola patients treated in the U.S. have been approved to treat the flu, the thinking is that, even if it turns out to be ineffective, it won’t do any harm.

Doctors Without Borders will also be conducting clinical trials of the antiviral brincidofovir in West Africa this month. Brincidofovir, which is made by the biotech firm Chimerix, is currently in Phase III clinical trials of favipiravir in West Africa this month. Since the drug has already
The compound works by inhibiting viral DNA polymerase. Ebola doesn’t have DNA—it has RNA—making any effectiveness of the drug against Ebola something of a mystery. Brincidofovir is actually a prodrug of cidofovir, a compound that’s been shown to be ineffective against RNA viruses, Chandran points out. But when the Centers for Disease Control & Prevention screened brincidofovir in a cell culture of human Ebola infection, it turned out to be remarkably effective.

“It clearly inhibits the virus in cells, but lots of things inhibit the virus in cells. Half of the Sigma catalog does,” Chandran says. “The question that really needs to be answered is, Is this actually effective in a therapeutic setting? The fact is that we don’t really know how it works or if it works.”

But Sarepta found that L poses a problem. It’s the first gene copied during transcription, and as a result, more copies of L tend to be made than are made of the other proteins. It was too tough to take out all of it. “Even if you knock down 90%, you can still produce quite a bit of virus,” Iversen notes.

Sarepta worked around the problem by putting one oligo targeting L, along with AVI-7537, which blocks the transcript for VP24. The resulting drug was AVI-7537, which blocks the transcript for VP24.

More recent research has helped explain why VP24 turned out to be the best target for Sarepta’s antisense program, Iversen says. Scientists have found that VP24 is able to disrupt the activity of a signaling protein called STAT1, which turns on a host of genes that regulate interferons, the body’s natural defense against viruses. “Essentially the virus is in some measure crippling the immune response,” Iversen says.

AVI-7537 showed promise in monkeys in an early human clinical trial, but in 2012, in the face of budget cutbacks, the Department of Defense stopped funding the project. The company is now trying to revive it. “We are actively pursuing funding opportunities both within the U.S. and externally with philanthropic foundations,” Iversen says.

The most promising targets were the transcripts for three proteins: VP24, VP35, and L. In the early 2000s, conventional wisdom among virologists was that L, which contains the genetic code for viral polymerase, might be the most vulnerable spot in the Ebola genome.

When the company skipped testing its oligonucleotides to block RNA transcripts, or copies, is fairly straightforward, the company took an empirical approach to figuring out which ones would make the most sense as a drug.

“Basically, because there are only seven genes, we targeted all seven,” says Patrick Iversen, a Sarepta scientist who has worked on the company’s Ebola program since its inception in the early 2000s.

The company skipped testing its oligonucleotides in cell culture and went straight to putting them in animals, conducting dozens of studies in mice to understand the effects of blocking the production of individual proteins and protein combinations.

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Whereas antivirals disrupt transcription altogether, other treatments are trying to stop snippets of RNA from doing their job. Two companies are using RNAi-based technologies—either single- or double-stranded RNA—to prevent key proteins from being manufactured.

Sarepta Therapeutics has spent more than a decade working on the Ebola virus, with much of that research done in partnership with USAMRIID. Because designing oligonucleotides to block RNA transcripts, or copies, is fairly straightforward, the company took an empirical approach to figuring out which ones would make the most sense as a drug.

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**EMERGENCY EBOLA FUNDS**

- **$6.2 billion** requested by President Obama on Nov. 5
- **$2.9 billion** for the State Department and USAID to fight Ebola in West Africa
- **$2.4 billion** for HHS (plus a $751 million contingency fund)
- **$112 million** for DOD to develop technologies to fight Ebola and shorten vaccine development
- **$25 million** for FDA to evaluate safety and efficacy of Ebola drugs and vaccines
- **$157 million** for BARDA to develop vaccines and therapeutics
- **$238 million** for NIH, including $56 million to fund Phase II and III trials of vaccines and therapeutics in Liberia
- **$606 million** to strengthen global health security, build health infrastructure in Ebola-susceptible countries
- **$603 million** for CDC public health efforts in West Africa
- **$787 million** for CDC/ASPR domestic preparedness and response efforts

**NOTE:** Congress is likely to approve the Ebola request as part of a spending package to keep the federal government funded beyond Dec. 11, 2014, when the current continuing resolution expires.

**KEY:** CDC = Centers for Disease Control & Prevention, ASPR = Office of the Assistant Secretary for Preparedness & Response, NIH = National Institutes of Health, BARDA = Biomedical Advanced Research & Development Authority, DOD = Department of Defense, HHS = Department of Health & Human Services, USAID = U.S. Agency for International Development.
Researchers Get Ready To Test Two Vaccines In West Africa

While many scientists are hard at work to find therapies to treat Ebola patients, other researchers are concentrating their efforts on creating vaccines to keep the virus from infecting people in the first place.

Scientists have searched for an Ebola vaccine since the first outbreak of the disease in 1976. Injections of inactivated virus proved to be ineffective in nonhuman primates. Over the intervening decades a number of classically vaccine approaches were tried, but none proved effective, and funding for such research has been scarce.

In recent months, two different Ebola vaccines—ChAd3 and VSV-EBOV—have undergone Phase I trials in healthy human volunteers to determine whether they are safe and what their appropriate doses are. Larger trials of the vaccines’ efficacy could begin as early as this month in Sierra Leone and Liberia, two Ebola-stricken countries in West Africa. People expected to get the experimental vaccines as part of the larger trial include health care workers.

Scientists at the National Institute of Allergy & Infectious Diseases (NIAID) originally developed ChAd3 in collaboration with the biotech company Okairos. GlaxoSmithKline (GSK) bought Okairos and its Ebola vaccine last year.

ChAd3 is made from an adenovirus that affects chimpanzees. The adenovirus acts as a carrier, delivering the gene for making the Ebola glycoprotein, which decorates the surface of Ebola virus particles and is critical to infection. The modified adenovirus prompts the vaccine recipient’s cells to express the glycoprotein; subsequently, the body mounts an immune response to it. Because the adenovirus does not replicate, researchers anticipate that the ChAd3 vaccine will require a booster.

VSV-EBOV, a vaccine developed by the Public Health Agency of Canada and licensed last week by Merck & Co. from NewLink Genetics, does replicate, so researchers believe it can work with just one jab. It uses vesicular stomatitis virus, or VSV, a virus that affects livestock. Scientists swap VSV’s glycoprotein gene with the gene for making the Ebola glycoprotein. As with ChAd3, the vaccine recipient starts making the Ebola glycoprotein and then develops an immune response to it. Even with large-scale trials starting for these two vaccines, doctors and scientists face plenty of challenges. Andrea Marzi, a virologist who studies Ebola at NIAID, notes that in West Africa, where access to low-temperature storage is limited, it will be tough to keep these vaccines at –80 °C, a temperature required to sustain activity.

Manufacturing presents another hurdle. “One challenge is finding sufficient sterile capacity for filling and packaging finished vials,” GSK spokeswoman Mary Anne Rhyne says. “Because Ebola vaccines use genetically modified organisms, the sterile filling of vials must be done in a biosafety level 2 facility, which only a few companies possess.”

Because of the crisis, Rhyne adds, GSK is taking the unusual step of ramping up production before it even knows if its vaccine is safe and effective.—BETHANY HALFORD
be analyzed for signs of a treatment’s efficacy; if an investigational drug looks good, even at an early checkpoint, everyone in the study will be given it. Cox believes this novel trial design will “allow a winner to be declared very early.”

Other practical realities will influence what the “right” drug is for this outbreak. “To a certain extent, a lot depends on the right drug being available early enough for us to be able to study it before the outbreak ends,” Sprecher says. ZMapp, for example, is grown in tobacco plants, a process that takes months.

If enough material for a clinical study isn’t available until later in 2015, the outbreak may have abated and there might not be enough sick people to test. That sounds like a good problem to have, but everyone from basic researchers to relief groups to government agencies wants to figure out an answer to avoid future crises. “It would be really nice to not have to go through this again,” Sprecher says.

Researchers, meanwhile, stress the need for ongoing funding to support Ebola research, even after this outbreak has died out.

**EBOLA TESTING**

**Prompted By Government, Companies Pursue Simple, Rapid Diagnostics**

The race is on for a reliable, low-cost test that can rapidly detect whether a person is infected with the Ebola virus. Ideally, such a test could be performed where patients are being treated for Ebola, or in airports where passengers are being screened, using only a drop of blood, saliva, or urine.

The current gold standard for confirming that a person has contracted Ebola is reverse transcriptase polymerase chain reaction, or RT-PCR. The method, which involves amplifying and detecting viral nucleic acid material, requires a full test tube of blood and costs about $100 per sample. Results can be confirmed in two to six hours once a sample arrives at a laboratory, but in West Africa, where the Ebola outbreak is centered, it can take several days for samples to reach those labs.

Since October, the U.S. Food & Drug Administration has authorized the emergency use of six diagnostics for Ebola, all of which rely on RT-PCR. Although RT-PCR is accurate when performed by skilled technicians on sophisticated machines, it is not well suited for use in countries where laboratories are few and far between.

A faster, easier-to-use diagnostic test is critical to containing the current Ebola outbreak, according to the World Health Organization (WHO), the public health arm of the United Nations. Such tests are needed to confirm suspected cases, guide clinical decisions, aid contact tracing, and facilitate early detection of new cases, WHO says.

Companies showed little interest in developing Ebola diagnostics before the current crisis in West Africa because demand for them was scant. But recent efforts by WHO, the U.S. Agency for Internationa...
down. “If we want these therapies and vaccines, we have to put in the legwork and do it over a long period of time, when nobody is watching, not just when there is public interest,” Einstein’s Chandran says. “Right now there is a clamoring, ‘Give me everything you have. Open your cupboards.’ It doesn’t work like that. It takes years to develop a drug.”

day before a committee of experts last month to evaluate the best proposals. Some of the winning technologies may be in the field within months, according to testimony presented by USAID Administrator Rajiv Shah at a congressional hearing in mid-November.

Government efforts, as well as heightened awareness of the situation in West Africa, have prompted numerous companies to begin working on rapid Ebola tests that rely on antigen detection. It remains to be seen, however, whether such tests will be finalized in time to make a difference in the current outbreak.

One of the tests is being developed by Corgenix Medical and members of an academic-industry partnership called the Viral Hemorrhagic Fever Consortium and led by Tulane University. Earlier this year, the group began developing the test, which relies on antibodies that bind to proteins of the Ebola virus. The work is being supported by a three-year, $2.9 million grant from the National Institutes of Health. Corgenix hopes the test will be able to detect Ebola from a drop of blood on a test strip in about 15 minutes.

The company emphasizes, however, that the test has not been finalized.

“This is a new three-year project, building off work commenced in 2010. We do not currently have an Ebola test, nor will we likely have a final one in the near future,” Corgenix CEO Douglass T. Simpson said in response to inaccurate media reports in August that the company’s device was nearly ready for prime time.

Corgenix and other companies say they are doing everything they can to get a reliable and rapid diagnostic kit for Ebola into West Africa. Although such tests may not be available in time for the current outbreak, company officials are hopeful that they will be ready for the next one.—BRITT ERICKSON