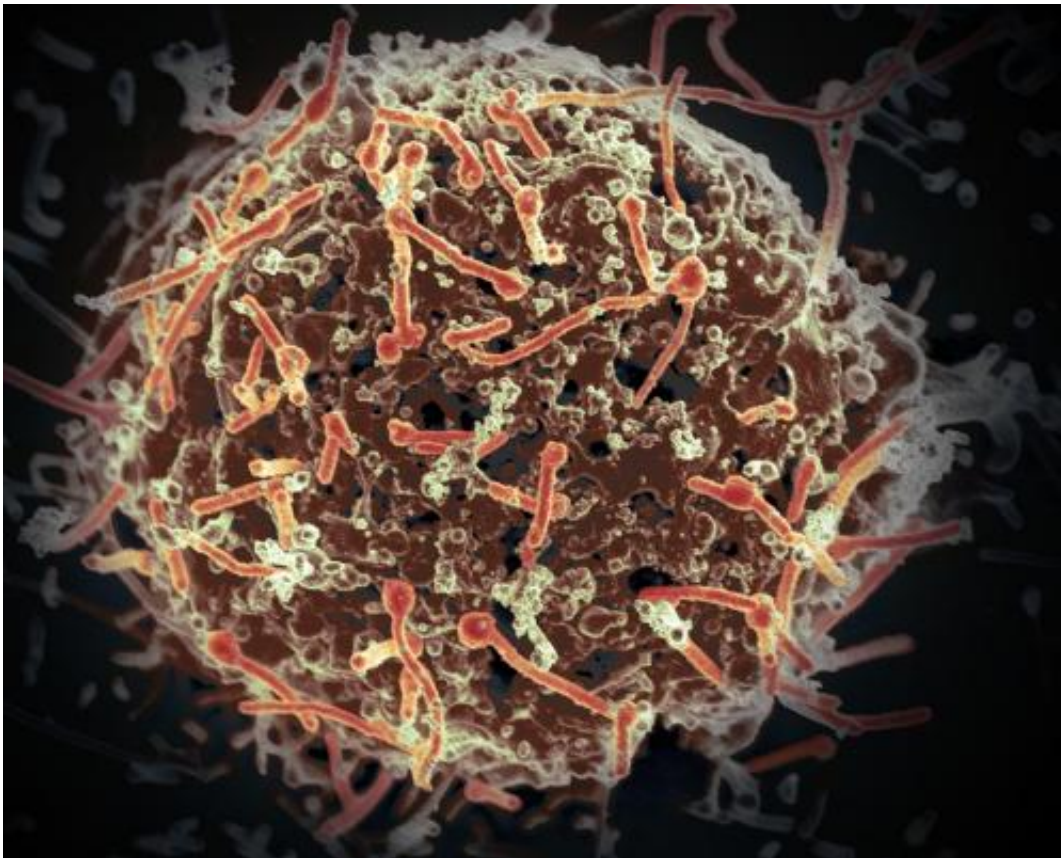# Drug combination defeats dengue, Ebola in mice, study finds

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The Ebola virus, isolated in November 2014 from patient blood samples obtained in Mali. The virus was isolated on Vero cells in a BSL-4 suite at Rocky Mountain Laboratories. Credit: NIAID

A combination of two cancer drugs inhibited both dengue and Ebola virus infections in mice in a study led by Stanford University School of

Medicine researchers, despite the fact that these two viruses are vastly different from each other.

In laboratory-dish experiments, the drug combination, which has previously shown efficacy against the hepatitis C virus, also was effective against West Nile and Zika viruses, both of which are relatives of the hepatitis C virus, and multiple other unrelated viruses.

The multi-institution study, to be published online Feb. 27 in the *Journal of Clinical Investigation*, also pinpointed the specific molecular mechanism by which these drugs derail a variety of RNA viruses, whose genetic material consists not of DNA but of its close relative, RNA.

"We've shown that a single combination of drugs can be effective across a broad range of viruses—even when those viruses hail from widely separated branches of the evolutionary tree," said the study's senior author, Shirit Einav, MD, assistant professor of infectious diseases and of microbiology and immunology.

The study's lead authors are former Stanford postdoctoral scholars Elena Bekerman, PhD, now at Gilead Sciences Inc., and Gregory Neveu, PhD, now at the University of Lyon and French National Institute of Health and Medical Research.

The reason the drugs used in the study are able to combat infections by such different viruses is that their disabling action is directed not at the virus but at proteins of the host cell it's trying to infect, Einav said.

The challenges posed by RNA viruses

Einav and her team are investigating strategies for combatting RNA viruses, such as dengue and Ebola. These viruses have a faulty replication process that results in frequent errors as their genetic material

is copied, rendering them especially prone to mutations. Consequently, they swiftly acquire resistance to a typical antiviral drug that targets a specific viral enzyme, Einav said.

"The 'one drug, one bug' approach can be quite successful, as in the case of hepatitis C virus," for which a concerted effort has generated several approved antiviral treatments, she said. But it took more than 10 years of research, she noted, and drug development costs typically exceed $2 billion. Making matters worse, Einav added, is the impossibility of predicting what the next emerging viral threat will look like.

"We're always getting blindsided," she said.

The deadly Ebola epidemic of a few years ago has subsided but could return at any time. Dengue infects an estimated 390 million people annually in over 100 countries. Four distinct strains of the dengue virus exist, hampering the development of a vaccine and boosting the chances of a once-infected person's re-infection by a different strain against which that person hasn't achieved sufficient immunity. Secondary infections can become life-threatening.

While an Ebola vaccine has shown promise, it's not yet approved. A recently approved dengue vaccine has only limited efficacy. No viable antiviral drugs are currently available for either virus.

Picking a different target

Viruses are cut-rate brigands: They produce nothing on their own, but rather hijack the machinery of our cells. Hepatitis C, dengue, Ebola and other viruses hop onto molecular "buses" that whisk cargo between cell compartments. These buses shuttle the viruses around inside of cells. The buses' routes and fares are regulated by numerous cellular enzymes. Two such enzymes, which go by the acronyms AAK1 and GAK,

essentially lower the fares charged by the molecular buses by tweaking them so they bind more strongly to their cargo.

The standard antiviral approach aims to disable a specific viral enzyme. Einav and her associates' alternative approach took advantage of viruses' total dependence on infected cells' molecular machinery.

The two-drug drug combination Einav's team put to work against dengue and Ebola impedes AAK1's and GAK's activity, effectively pricing bus fares beyond the viral budget. Erlotinib and sunitinib, each approved by the Food and Drug Administration more than a decade ago, are prescribed for various cancer indications. Neither AAK1 nor GAK are the primary targets of these drugs in their cancer-fighting roles. But Einav's group discovered, by accessing publicly available databases, that the two drugs impair AAK1 and GAK activity, too.

Einav and her colleagues previously demonstrated that erlotinib and sunitinib inhibit hepatitis C virus infection in cells. In the new study, the investigators conducted experiments in lab dishes to show that both drugs inhibit viral infection by impeding the activity of AAK1 and GAK.

Next, they tested the combination in lab dishes against the dengue and Ebola viruses, and observed that viral activity was strongly inhibited in both. While the dengue virus is a relatively close cousin of hepatitis C, it is quite different from the Ebola virus. The same drug combination also showed efficacy against a variety of other RNA viruses related to hepatitis C, including the Zika and West Nile viruses, and even against several unrelated viruses.

Testing the combination in mice

In a prevention experiment in mice, the investigators administered the

erlotinib-sunitinib combination once daily starting on the day of dengue-virus infection, employing the two drugs for five days at doses comparable to those approved for use against cancer in humans. All the control mice died between days four and eight. But of those treated with the drug combination, 65 to 100 percent, depending on the individual experiment, survived and regained their pre-infection weight and mobility. Given individually, the drugs provided substantially less protection, Einav said.

In another experiment designed to test the drugs as a therapy, the combination retained substantial antiviral efficacy as long as it was given less than 48 hours after infection.

In a similar prevention experiment with the Ebola virus, the scientists administered the drug daily for 10 days starting at six hours before infection. Some 90 percent of the control mice died within a week or two. But half the mice receiving the drug combination survived. Again, the drugs were substantially less effective when given individually.

Additional lab experiments showed that the combination profoundly inhibited the dengue virus's ability to develop drug resistance. There's no possible way for viral mutations to alter the proteins of the cells it infects, Einav said, and no easy way for the virus to mutate around its dependence on those proteins.

Stanford's Office of Technology Licensing has filed for patents on intellectual property associated with the findings.

Provided by Stanford University Medical Center