Ebola Filovirus Drug and Vaccine Development

Tom Geisbert
Filoviruses: Taxonomy

Mononegavirales

Rhabdoviridae

Filoviridae

Paramyxoviridae

Marburgvirus

Ebolavirus

Nucleotide and amino acid differences between EBOV and MARV are ~ 55%

EBOV species show 37-41% difference in nucleotide and amino acid sequences

Marburgvirus

Ebolavirus

Zaire

Sudan

Reston

Ivory Coast

Bundibugyo
The NP and VP30 proteins of filoviruses are the major and minor nucleoproteins, respectively, and interact strongly with the genomic RNA molecule to form the viral NC (along with VP35 and L)

L and VP35 form the polymerase complex which acts to transcribe and replicate the filovirus genome

The VP40 protein functions as the matrix protein and the VP24 protein may have a secondary/minor matrix protein function

GP is the structural (surface) glycoprotein required for virus entry
Ebola and Marburg Virus Outbreaks

- ~1500 Fatal Cases of Ebola HF
- ~360 Fatal Cases of Marburg HF

Map showing outbreaks in countries such as DRC, Gabon, Angola, Zimbabwe, Sudan, and Uganda.
A total of 24,701 confirmed, probable, and suspected cases of Ebola virus disease (EVD) have been reported in seven affected countries (Guinea, Liberia, Sierra Leone, Mali, Senegal, Spain, the United Kingdom, and the United States of America) up to the end of 15 March 2015. There have been 10,194 deaths.
Infection of the Americans
Infection of the Americans
Infection of the Americans
A man who took a commercial flight from Liberia that landed in Dallas on Sept. 20 has been found to have the Ebola virus, the Centers for Disease Control and Prevention reported on Tuesday. He is the first traveler to have brought the virus to the United States on a passenger plane and the first in whom Ebola has been diagnosed outside of Africa in the current outbreak.

As the disease has swept across West Africa, many health experts said it would be only a matter of time before it reached the United States. Hospitals and health departments around the country have been preparing for it, and a number of false alarms have occurred. But this time, the case is real.
Thomas Eric Duncan: First Ebola death in U.S.

By Greg Botelho and Jacque Wilson, CNN
updated 3:19 PM EDT, Wed October 8, 2014

CNN) -- Thomas Eric Duncan left Liberia for the United States, by official accounts, a healthy man. Just over two weeks later, he passed away at a Dallas, Texas, hospital with Ebola.

Duncan was admitted into isolation at Texas Health Presbyterian Hospital on September 28 with common symptoms of Ebola: fever, vomiting and diarrhea. He later tested positive for the virus that has killed more than 3,400 people in West Africa.

He was started on the experimental drug brincidofovir on October 4 -- far too long after he arrived at the hospital, his family has said. On Tuesday, the hospital reported that Duncan was on a ventilator and his kidneys were failing.
Dr. Geisbert, So why don’t we have a vaccine?
Approaches to Filovirus Vaccines

- Inactivated antigens
  - Whole virion preparations
  - Liposomes
  - Rhabdovirus

- DNA

- Virus-like particles

- Replication-defective vectors
  - Vaccinia
  - VEEV replicons
  - Adenovirus

- Replicating vectors
  - Vesicular stomatitis virus
  - Paramyxovirus
  - Rhabdovirus

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**The New York Times**

Vaccine Shows Promise for Fighting Ebola Virus

By DENISE GRADY

Scientists trying to develop vaccines against Africa's deadly Marburg and Ebola viruses are reporting an important milestone, a new type of vaccine that prevents the diseases in

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**BREAKTHROUGH!**

Scientists have developed vaccines effective in monkeys against both the Marburg and Ebola viruses

By JAMES RAZA | News-Post Staff | jraza@newyorktimes.com

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FRIDAY, March 10, 2017 — Last fall, after decades of civil war, Angola faced a new enemy. This one was unseen and killed from within.

The new enemy is the Marburg virus. It can kill 90 percent of its infected victims quickly and painfu. It can cause internal organs to liquefy, skin to bubble up, and victims to weep tears of blood, according to Richard Preston in his book "The Hot Zone." More than 225 people have been infected since the Marburg virus outbreak began last fall. While very little can be done to stop this outbreak, future "hot zones" may see some cooling with help from Frederick and Winnipeg, Canada.

Scientists from the U.S. Army Medical Research Institute of Infectious Diseases and the Public Health Agency of Canada have developed the first vaccines against both the Marburg and Ebola viruses that protect monkeys.

"We've made more progress in the past three years than we probably made in the previous 30," said Dr. Thomas Geisbert, with the U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick.

Mr. Geisbert, along with Canadian researchers Dr. Heinz Feldmann and Dr. Steven Jones of PHAC's National Microbiology Laboratory, developed the vaccines after years of work on the problem. This month's Nature Medicine journal published their study.

"When you see the tragedies these viruses cause, it's very frustrating that we can't do more to help people," said Mr. Feldmann, who has been providing on-site rapid diagnostic support to the cur..."
## Success of Vaccines Against Filoviruses in NHPs

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. of Inj’s</th>
<th>MARV- Angola</th>
<th>MARV- Musoke</th>
<th>Ebola- Zaire</th>
<th>Ebola- Sudan</th>
<th>Ebola- IC</th>
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<tr>
<td>DNA</td>
<td>4</td>
<td>Yes</td>
<td>NR</td>
<td>No</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>DNA prime/Ad5 boost</td>
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<td>NR</td>
<td>Yes</td>
<td>NR</td>
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<td>NR</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
</tr>
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<td>Chimpanzee adenovirus (ChAd3)</td>
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<td>NR</td>
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<td>NR</td>
<td>NR</td>
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<tr>
<td>Vesicular stomatitis virus (VSV), deltaG + N4CT1</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Virus Like Particles (VLPs)</td>
<td>3</td>
<td>NR</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Alphavirus replicons (VEEV replicons)</td>
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<td>Yes (3)</td>
<td>Yes (1)</td>
<td>Yes (2)</td>
<td>NR</td>
</tr>
<tr>
<td>Rhabdovirus (Rabies)</td>
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<td>NR</td>
<td>Variable</td>
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<td>NR</td>
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<tr>
<td>Human parainfluenza virus type 3 (HPIV3)</td>
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<td>NR</td>
<td>NR</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

GP, NP, and VP40 have been used as immunogens; however, GP is the primary immunogen and can provide protection alone.
The Politics of Ebola Vaccines and Treatments

- Small Global Market
- No Financial Incentive for Big Pharma
- The “FDA Animal Rule”

Increased funds were available post 9/11 from US Government agencies to develop countermeasures against Ebola. However, funds not sufficient for Phase I trials and of equal importance there has been confusion regarding FDA Animal Rule.
Ebola Vaccines - Clinical Trials

**Outbreak has fast tracked vaccines**

- GlaxoSmithKline (GSK), ChAd3
- NewLink Genetics/Merck, VSV-delta G
- Profectus Biosciences, VSV-N4CT1
- Johnson & Johnson and Bavarian Nordic, Adenovirus and MVA
New Ebola vaccine study has begun in Maryland

Researchers have started testing two vaccines, but until they're ready, here's a look at experimental therapies being used to battle the virus. (USA NEWS, USA TODAY)

A VOLUNTEER RECEIVES AN EBOLA VACCINATION IN BAMAKO, MALI. (PHOTO: ALEX DUVAL SMITH, EPA)

The first human trials of a Canadian Ebola vaccine began Monday, part of a flood of experimental therapies rushed into testing to battle the Ebola epidemic.
Ebola Vaccines - Clinical Trials

- GlaxoSmithKline (GSK), ChAd3
- NewLink Genetics/Merck, VSV-delta G
- Profectus Biosciences, VSV-N4CT1
- Johnson & Johnson and Bavarian Nordic, Adenovirus and MVA
Vesicular Stomatitis Virus (VSV) Recombinants

VSV-wt

VSVΔG

VSVΔG-EBOVGP

VSVΔG-MARVGP

Ebola GP

Marburg GP
Attenuation of Recombinant Vesicular Stomatitis Virus-Human Immunodeficiency Virus Type 1 Vaccine Vectors by Gene Translocations and G Gene Truncation Reduces Neurovirulence and Enhances Immunogenicity in Mice

David Cooper,* Kevin J. Wright, Priscilla C. Calderon, Min Guo, Farooq Nasar, J. Erik Johnson, John W. Coleman, Margaret Lee, Cheryl Kotash, Irene Yurgelonis, Robert J. Natuk, R. Michael Hendry, Stephen A. Udem,† and David K. Clarke

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Received 10 July 2007/Accepted 7 October 2007

Recombinant vesicular stomatitis virus (rVSV) has shown great potential as a new viral vector for vaccination. However, the prototypic rVSV vector described previously was found to be insufficiently attenuated for clinical evaluation when assessed for neurovirulence in nonhuman primates. Here, we describe the attenuation, neurovirulence, and immunogenicity of rVSV vectors expressing human immunodeficiency virus type 1 Gag. These rVSV vectors were attenuated by combinations of the following manipulations: N gene translocations (N4), G gene truncations (CT1 or CT9), noncytopathic M gene mutations (Mncp), and positioning of the gag gene into the first position of the viral genome (gag1). The resulting N4CT1-gag1, N4CT9-gag1, and MncpCT1-gag1 vectors demonstrated dramatically reduced neurovirulence in mice following direct intracranial inoculation. Surprisingly, in spite of a very high level of attenuation, the N4CT1-gag1 and N4CT9-gag1 vectors generated robust Gag-specific immune responses following intramuscular immunization that were equivalent to or greater than immune responses generated by the more virulent prototypic vectors. MncpCT1-gag1 also induced Gag-specific immune responses following intramuscular immunization that were equivalent to immune responses generated by the prototypic rVSV vector. Placement of the gag gene in the first position of the VSV genome was associated with increased in vitro expression of Gag protein, in vivo expression of Gag mRNA, and enhanced immunogenicity of the vector. These findings demonstrate that through directed manipulation of the rVSV genome, vectors that have reduced neurovirulence and enhanced immunogenicity can be made.
1. **Durability.** Nearly all filovirus vaccines have been evaluated in NHPs against virus challenge within 4-8 weeks after the final vaccination.

2. **Potency of the virus challenge stock.** New data suggests that *Zaire ebolavirus* (ZEBOV) seed stocks with high populations of viruses with wild type “7U” residues at the GP editing may be more pathogenic than mutant viruses with “8U” residues.

3. **Protection against multiple species and strains of filoviruses including ZEBOV Makona**
Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge


Ebolavirus disease causes high mortality, and the current outbreak has spread unabated through West Africa. Human adenovirus type 5 vectors (rAd5) encoding ebolavirus glycoprotein (GP) generate protective immunity against acute lethal Zaire ebolavirus (EBOV) challenge in macaques, but fail to protect animals immune to Ad5, suggesting natural Ad5 exposure may limit vaccine efficacy in humans. Here we show that a chimpanzee-derived replication-defective adenovirus (ChAd) vaccine also rapidly induced uniform protection against acute lethal EBOV challenge in macaques. Because protection waned over several months, we boosted ChAd3 with modified vaccinia Ankara (MVA) and generated, for the first time, durable protection against lethal EBOV challenge.

Human-derived rAd vectors have undergone extensive development in proof-of-concept and clinical trials as vaccines for multiple pathogens. For ebolavirus, rAd vaccines that confer protection against acute EBOV challenge generate effector CD8+ T-cell responses within 3 weeks of immunization and therefore have the potential to provide rapid immunity in an acute human outbreak setting. rAd5 EBOV vaccines protect against acute EBOV challenge (4 weeks after vaccination), whereas EBOV vaccines using alternative human adenoviruses do not, perhaps owing to differences in antigen expression or target cell receptor preference. However, human-derived rAd vectors are limited by preexisting immunity to the vectors. Adenoviruses isolated from nonhuman sources including chimpanzees and apes may overcome this limitation, and they have shown promise for EBOV protection, but only against a modified virus in mice. ChAd5 have been evaluated as vaccine vectors in mice, nonhuman primates (NHPs) and human clinical trials, but have low worldwide seroprevalence and are not cross-neutralized by human anti-adenovirus sera. Here we evaluated monovalent (EBOV)

**Table 1 Durable vaccine protection against EBOV**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Dose (PU)</th>
<th>Protection^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single shot</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChAd3</td>
<td>$1 \times 10^{11}$</td>
<td>2/4</td>
</tr>
<tr>
<td>ChAd3</td>
<td>$1 \times 10^{10}$</td>
<td>0/4</td>
</tr>
<tr>
<td><strong>Prime-boost</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChAd3/ChAd3</td>
<td>$1 \times 10^{10}/1 \times 10^{10}$</td>
<td>1/3</td>
</tr>
<tr>
<td>ChAd3/ChAd63</td>
<td>$1 \times 10^{10}/1 \times 10^{10}$</td>
<td>1/4</td>
</tr>
<tr>
<td>ChAd3/MVA</td>
<td>$1 \times 10^{10}/1 \times 10^{8}$</td>
<td>4/4</td>
</tr>
</tbody>
</table>

Animals in single-shot groups were vaccinated with ChAd3 at the doses indicated and exposed to a lethal dose of EBOV 10 months after the prime vaccination. Animals in prime-boost groups were primed with ChAd3, boosted 8 weeks later and exposed to a lethal dose of EBOV as in the single-shot groups.

^aProtection is shown as the number surviving/total challenged and as a survival percentage.
VSV-Based Vaccines (delta G) - Durability

Durability of a Vesicular Stomatitis Virus-Based Marburg Virus Vaccine in Nonhuman Primates

Chad E. Mire¹ ², Joan B. Geisbert¹ ², Krystle N. Agans¹ ², Benjamin A. Satterfield¹ ², Krista M. Versteeg¹ ², Elizabeth A. Fritz¹ ², Heinz Feldmann³, Lisa E. Hensley⁴, Thomas W. Geisbert¹ ²*

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Abstract

The filoviruses, Marburg virus (MARV) and Ebola virus, causes severe hemorrhagic fever with high mortality in humans and nonhuman primates. A promising filovirus vaccine under development is based on a recombinant vesicular stomatitis virus (rVSV) that expresses individual filovirus glycoproteins (GP) in place of the VSV glycoprotein (G). These vaccines have shown 100% efficacy against filovirus infection in nonhuman primates when challenge occurs 28-35 days after a single injection immunization. Here, we examined the ability of a rVSV MARV-GP vaccine to provide protection when challenge occurs more than a year after vaccination. Cynomolgus macaques were immunized with rVSV-MARV-GP and challenged with MARV approximately 14 months after vaccination. Immunization resulted in the vaccine cohort of six animals having anti-MARV GP IgG throughout the pre-challenge period. Following MARV challenge none of the vaccinated animals showed any signs of clinical disease or viremia and all were completely protected from MARV infection. Two unvaccinated control animals exhibited signs consistent with MARV infection and both succumbed. Importantly, these data are the first to show 100% protective efficacy against any high dose filovirus challenge beyond 8 weeks after final vaccination. These findings demonstrate the durability of VSV-based filovirus vaccines.


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Competing Interests: CEM, JBG, KNA, BAS, KMV, EAF, and LEH have declared that no competing interests exist. TWG and HF claim intellectual property regarding VSV-based vaccines for the prevention and treatment of filovirus infections. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

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Gaps and Questions

1. Durability. Nearly all filovirus vaccines have been evaluated in NHPs against virus challenge within 4-8 weeks after the final vaccination.

2. Potency of the virus challenge stock. New data suggests that *Zaire ebolavirus* (ZEBOV) seed stocks with high populations of viruses with wild type “7U” residues at the GP editing may be more pathogenic than mutant viruses with “8U” residues.

3. Protection against multiple species and strains of filoviruses including ZEBOV Makona
Two mRNA species are synthesized from the Ebola GP gene as a result of transcriptional editing, which leads to the insertion by the viral polymerase of an extra adenosine at a specific-editing site near the middle of the coding region. This site consists of 7 consecutive uridines in the genomic sequence. The structural membrane anchored GP is expressed only when an extra (eighth) adenosine is inserted into the nascent mRNA via stuttering of the EBOV polymerase over the editing site. The EBOV polymerase transcribes the GP gene with fidelity ~ 80% of the time, and these unedited mRNAs program the expression of the predominant GP gene product, sGP, a nonstructural, secreted glycoprotein.
Both studies show that passage of ZEBOV/7U in Vero E6 cells results in the appearance and rapid accumulation of a variant (ZEBOV/8U) containing an additional uridine at the editing site in the viral genome. EBOV/8U outgrows and eventually replaces the wild-type EBOV during passage in Vero E6 cells.
Function of Ebola sGP?

Release of Viral Glycoproteins during Ebola Virus Infection
Viktor E. Volchkov,¹ Valentina A. Volchkova, Werner Stenczka, Hans-Dieter Klenk, and Heinz Feldmann
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Received December 16, 1997; returned to author for revision January 22, 1998; accepted March 11, 1998

Antigenic Subversion: A Novel Mechanism of Host Immune Evasion by Ebola Virus
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Abstract
In addition to its surface glycoprotein (GP₁₂), Ebola virus (EBOV) directs the production of large quantities of a truncated glycoprotein isoform (sGP) that is secreted into the extracellular space. The generation of secreted antigens has been studied in several viruses and suggested as a mechanism of host immune evasion through absorption of antibodies and interference with antibody-mediated clearance. However, such a role has not been conclusively determined for the Ebola virus sGP. In this study, we immunized mice with DNA constructs expressing GP₁₂ and/or sGP, and demonstrate that sGP can efficiently compete for anti-GP₁₂ antibodies, but only from mice that have been immunized by sGP. We term this phenomenon “antigenic subversion”, and propose a model whereby sGP redirects the host antibody response to focus on epitopes which it shares with membrane-bound GP₁₂, thereby allowing it to absorb anti-GP₁₂ antibodies. Unexpectedly, we found that sGP can also subvert a previously immunized host’s anti-GP₁₂ response resulting in strong cross-reactivity with sGP. This finding is particularly relevant to EBOV vaccinology since it underscores the importance of eliciting robust immunity that is sufficient to rapidly clear an infection before antigenic subversion can occur. Antigenic subversion represents a novel virus escape strategy that likely helps EBOV evade host immunity, and may represent an important obstacle to EBOV vaccine design.

Author Summary
The function of the Ebola virus (EBOV) secreted glycoprotein (sGP) has been long debated, and the fact that sGP production is conserved among all known EBOV species strongly indicates an important role in the viral life cycle. Furthermore, the recent finding that EBOV mutates to a predominantly non-sGP-forming phenotype in cell culture, while the mutant virus reverts to an sGP-forming phenotype in vivo, suggests that sGP is critical for EBOV to survive in its infected host. Here we demonstrate that sGP can function to absorb anti-GP antibodies. More importantly, instead of simply passively absorbing host antibodies, sGP actively subverts the host immune response to induce cross-reactivity with epitopes it shares with membrane-bound GP₁₂. Immune subversion by sGP represents a distinct mechanism from the use of secreted antigens as antibody decoys, an immune evasion tactic previously proposed for other viruses, and should be an important consideration for future EBOV vaccine design efforts since vaccines may need to be specifically tailored to avoid subversion.
Gaps and Questions

1. Durability. Nearly all filovirus vaccines have been evaluated in NHPs against virus challenge within 4-8 weeks after the final vaccination.

2. Potency of the virus challenge stock. New data suggests that Zaire ebolavirus (ZEBOV) seed stocks with high populations of viruses with wild type “7U” residues at the GP editing may be more pathogenic than mutant viruses with “8U” residues.

3. Protection against multiple species and strains of filoviruses including ZEBOV Makona
Antiviral Approaches

• Neutralization of virus; Passive transfer of antibodies (ZMapp)
• Inhibition of membrane fusion
• Inhibition of transcription and replication
  - Nucleoside analogues, Ribavirin
  - Antisense oligonucleotides and siRNA
  - Interference with viral assembly, maturation, and budding

Postexposure Vaccination (rVSV)

Modulation of the host immune response

• Interferons
• Regulation of coagulation
Ebola Hemorrhagic Fever: Evaluation of Passive Immunotherapy in Nonhuman Primates

Peter B. Jahrling,1 Joan B. Geisbert,2 James R. Swaremogen,1 Thomas Larsen,1 and Thomas W. Geisbert1

1Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, and Virology and Pathology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, and 2Association for Assessment and Accreditation of Laboratory Animal Care International, Rockville, Maryland

The survival of 7 of 8 patients with Ebola virus (EBOV) infection after transfusions of convalescent-phase blood during a 1995 outbreak of EBOV infection is frequently cited as evidence that passive immunotherapy is a viable treatment option. To test whether whole-blood transfusions were more efficacious than passively administered immunoglobulins or monoclonal antibodies, we transfused convalescent-phase blood from EBOV-immune monkeys into naive animals shortly after challenge with EBOV. Although passively acquired antibody titers comparable to those associated with effective vaccination were obtained, all monkeys that had received transfusions succumbed to infection concurrently with control monkeys. These data cast further doubt on the value of passive immunotherapy for the treatment of EBOV infection.

CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates

Nancy J Sullivan,1 Lisa Hensley,2 Clement Asiedu1, Thomas W Geisbert2,3, Daphne Stanley1, Joshua Johnson2, Anna Honko2, Gene Olinger2, Michael Bailey1,5, Joan B Geisbert2,3, Keith A Reimann3, Saran Bao4, Srinivas Rao1, Mario Roederer1, Peter B Jahrling6, Richard A Koup1 and Gary J Nabel1

Vaccine-induced immunity to Ebola virus infection in nonhuman primates (NHPs) is marked by potent antigen-specific cellular and humoral immune responses1,2; however, the immune mechanism of protection remains unknown. Here we define the immune basis of protection conferred by a highly protective recombinant adenovirus vector containing Ebola virus glycoprotein (GP)1,3 in NHPs. Passive transfer of high-titer polyclonal antibodies from vaccinated Ebola virus–immune cynomolgus macaques to naive macaques failed to confer protection against disease, suggesting a limited role of humoral immunity. In contrast, depletion of CD8+ T cells in vivo after vaccination and immediately before challenge eliminated immunity in two vaccinated macaques, indicating a crucial requirement for T cells in this setting. The protective effect was meditated largely by CD8+ cells, as depletion of CD8+ cells in vivo using the c-myc-T807 monoclonal antibody (mAb), which does not affect CD4+ T cell or humoral immune responses, abrogated protection in four out of five subjects. These findings indicate that CD8+ cells have a major role in rAd5-GP–induced immune protection against Ebola virus infection in NHPs. Understanding the immunologic mechanism of Ebola virus protection will facilitate the development of vaccines for Ebola and related hemorrhagic fever viruses in humans.
Postexposure Treatment Approaches

Antiviral Approaches

• Neutralization of virus; Passive transfer of antibodies (ZMapp)
• Inhibition of membrane fusion
• Inhibition of transcription and replication
  - Nucleoside analogues, Ribavirin
  - Antisense oligonucleotides and siRNA
  - Interference with viral assembly, maturation, and budding

Postexposure Vaccination (rVSV)

Modulation of the host immune response

• Interferons
• Regulation of coagulation
Favipiravir (T-705, Avigan)

A pyrazinecarboxamide derivative. Favipiravir is active against influenza viruses, West Nile virus, yellow fever virus, foot-and-mouth disease virus as well as other flaviviruses, arenaviruses, bunyaviruses and alphaviruses. Activity against enteroviruses and Rift Valley fever virus has also been demonstrated.

The mechanism of its actions is thought to be related to the selective inhibition of viral RNA-dependent RNA polymerase.
Brincidofovir is a modified version of an antiviral drug called cidofovir, which inhibits replication of a variety of DNA viruses including adenoviruses, poxviruses, and herpesviruses. When cidofovir enters a cell, two phosphates are added to the compound by a cellular enzyme, producing cidofovir diphosphate. Cidofovir is used by viral DNA polymerases because it looks very much like a normal building block of DNA, cytidine. For reasons that are not known, incorporation of phosphorylated cidofovir causes inefficient viral DNA synthesis. As a result, viral replication is inhibited.

Looking at the compound, one could not predict that it would inhibit Ebola virus, which has an RNA genome. RNA polymerases use different substrates than DNA polymerases.
A side effect of the improving status of the West Africa Ebola outbreak has led Chimerix to discontinue its participation in clinical efficacy trials of their lead antiviral drug, brincidofovir (CMX001; BCV).

On January 2, an open-label, Phase 2 study was initiated to evaluate brincidofovir’s efficacy in 140 Liberian patients with Ebola virus disease at Médecins Sans Frontières (MSF)’s ELWA 3 Ebola Management Centre in Monrovia, Liberia. The trial was led by investigators from the University of Oxford and the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) with MSF’s operational support and funding from The Wellcome Trust.

But with only four new cases of Ebola virus disease in Liberia last week, Chimerix said Friday that “only a handful of patients” were enrolled in the trial. Therefore, the company is withdrawing from the current, single-arm brincidofovir efficacy trial in Ebola virus disease and discontinuing discussions for future, randomized trials.
Postexposure Treatment Approaches

Antiviral Approaches

• Neutralization of virus; Passive transfer of antibodies (ZMapp)
• Inhibition of membrane fusion
• Inhibition of transcription and replication
  - Nucleoside analogues, Ribavirin
  - **Antisense oligonucleotides and siRNA**
  - Interference with viral assembly, maturation, and budding

Postexposure Vaccination (rVSV)

Modulation of the host immune response

• Interferons
• Regulation of coagulation
Small Interfering RNA (siRNA)

- Small interfering RNAs (siRNAs) are powerful, sequence-specific reagents designed to suppress the expression of genes through the process of RNA interference (RNAi).
- RNAi is an endogenous pathway involved in cellular defense against viral pathogens, transposons, and post-transcriptional regulation of endogenous transcripts.
- RNAi has been used in cell-culture systems to inhibit the replication of a number of viruses that cause diseases in humans (e.g., HIV, hep. C, poliovirus, SARS, Marburg).

- Difficulty in effective delivery of siRNAs in vivo has been the major obstacle to their use as therapeutic agents.
RNAi-mediated gene silencing in non-human primates

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Components

- Cationic Lipid
- Fusogenic Lipid
- PEG - Lipid
- Nucleic Acid

The stable nucleic acid lipid particle (SNALP) developed by Tekmira Pharmaceuticals consists of siRNA encapsulated in a lipid bilayer containing a diffusible polyethylene glycol (PEG)-lipid conjugate

siRNAs can silence ApoB

SNALP in Phase I trials for inhibiting tumor growth

Wheeler et al, Gene Therapy, 6:271-281, 1999
Small Interfering RNA (siRNA)

• Approach was to target the *Zaire ebolavirus* L protein as it provides polymerase activity and its suppression should lead to a loss of all viral RNA synthesis; siRNAs were designed to target individual regions of the *Zaire ebolavirus* L gene

- **EK1**
  - sense 5'-GUACGAAGCUGUAUAUAAdTdT-3'
  - antisense 5'-UUUAUAUACAGCUUCGUACdTdT-3'

- **EK2**
  - sense 5'-GGAUCUUGGUACAGUGUUAdTdT-3'
  - antisense 5'-UAACACUGUACCAAGAUCCdTdT-3'

- **EK3**
  - sense 5'-CAGGCUUAAUUCAGUUAAdTdT-3'
  - antisense 5'-UUUAACUGGAUAAGGCCUGdTdT-3'

- **EK4**
  - sense 5'-GUAAACGGCUGAACAUAUAUdTdT-3'
  - antisense 5'-AUAAUGUUCAGCCGUUUACdTdT-3'

• siRNAs were screened *in vitro* and then evaluated *in vivo*
# ZEBOV Targets and Target Sequences

Approach was to target ZEBOV VP24 and VP35 as both have been shown to have inhibitory effects on the host type I interferon (IFN) response

<table>
<thead>
<tr>
<th>Sequence Name</th>
<th>ZEBOV Target</th>
<th>Sequence</th>
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</table>
| VP24-775      | VP24         | sense 5’-GCUGAUUGACCAGUCUUUGAU-3’  
                |              | antisense 5’-CAAGACUGGUAACCUACUG-3’ |
| VP24-978      | VP24         | sense 5’-ACGGAUUGUUGAGCAGUAUUG-3’  
                |              | antisense 5’-AUACUGACUCAACAUCCGUUG-3’ |
| VP24-1160     | VP24         | sense 5’-UCCUCGACACGAAUGCAAGU-3’  
                |              | antisense 5’-UUUGCAUUGUGUGCAGGAUC-3’ |
| VP24-1160 mod | VP24         | sense 5’-UCCmUCGACACGAAmUGCAAGU-3’  
                |              | antisense 5’-UUmUGCAUUGUGUGCAGGAUC-3’ |
| VP35-219      | VP35         | sense 5’-GGGACAUCUUUGUAAUUG-3’     
                |              | antisense 5’-AAUACACAGAAAGUGUCUU-3’ |
| VP35-349      | VP35         | sense 5’-GGAGGUAGUACAAACAAUdGdTdT-3’ 
                |              | antisense 5’-CAUGUUUUGUACUACCUCdTdTdT-3’ |
| VP35-687      | VP35         | sense 5’-GGGAGGCAUUAACAAACUCU-3’  
                |              | antisense 5’-AGAUGGUAUGGAAUCCCUU-3’ |
| VP35-855      | VP35         | sense 5’-GCAACUCUUUGGAGCAUACU-3’  
                |              | antisense 5’-AUGAGUGGCAUACUGGAUGU-3’ |
| VP35-855 mod  | VP35         | sense 5’-GCAACmUCAUUGmGmGrArCrAmUCAU-3’ 
                |              | antisense 5’-AUGAUmGUCCAAUGAmGmUGCUA-3’ |
| Luc           | N/A          | sense 5’-GAUUAAUGGCUUAGUAAA-3’     
                |              | antisense 5’-UCACUAACCGGACAUAACU-3’ |
| Luc mod       | N/A          | sense 5’-GAmUmUAmUAmUGmUCCCGmUmUAmUAmUAAA-3’ 
                |              | antisense 5’-UACAmUAAACCGGACAmUAAmUCAU-3’ |

Note: 'm' in front of the base that designates a "2"-O-methyl (2’OMe) modification (unmodified versions do not have any 2’OMe modified bases)
Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study

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Summary
Background We previously showed that small interfering RNAs (siRNAs) targeting the Zaire Ebola virus (ZEBOV) RNA polymerase L protein formulated in stable nucleic acid-lipid particles (SNALPs) completely protected guineapigs when administered shortly after a lethal ZEBOV challenge. Although rodent models of ZEBOV infection are useful for screening prospective countermeasures, they are frequently not useful for prediction of efficacy in the more stringent non-human primate models. We therefore assessed the efficacy of modified non-immunostimulatory siRNAs in a uniformly lethal non-human primate model of ZEBOV haemorrhagic fever.

Methods A combination of modified siRNAs targeting the ZEBOV L polymerase (EK-1 mod), viral protein (VP) 24 (VP24-1160 mod), and VP35 (VP35-855 mod) were formulated in SNALPs. A group of macaques (n=3) was given these pooled anti-ZEBOV siRNAs (2 mg/kg per dose, bolus intravenous infusion) after 30 min, and on days 1, 3, and 5 after challenge with ZEBOV. A second group of macaques (n=4) was given the pooled anti-ZEBOV siRNAs after 30 min, and on days 1, 2, 3, 4, 5, and 6 after challenge with ZEBOV.

Findings Two (66%) of three rhesus monkeys given four postexposure treatments of the pooled anti-ZEBOV siRNAs were protected from lethal ZEBOV infection, whereas all macaques given seven postexposure treatments were protected. The treatment regimen in the second study was well tolerated with minor changes in liver enzymes that might have been related to viral infection.

Interpretation This complete postexposure protection against ZEBOV in non-human primates provides a model for the treatment of ZEBOV-induced haemorrhagic fever. These data show the potential of RNA interference as an effective postexposure treatment strategy for people infected with Ebola virus, and suggest that this strategy might also be useful for treatment of other emerging viral infections.
Ebola-Zaire Infection of Rhesus Macaques

Control

1000 PFU Zaire ebolavirus
Treatment of Ebola-Zaire-Infected Rhesus Macaques

Anti-ZEBOV L VP24 VP35 siRNAs

(2 mg/kg)

Control siRNA

Control
Treatment of Ebola-Zaire-Infected Rhesus Macaques

- Treatment started 30 min post-infection with ZEBOV VP24 VP35 siRNAs.
- Days 1-6: Administration of Anti-ZEBOV siRNA (2 mg/kg).
- Day 10: Control group administered with Control siRNA.
- No control group survival, indicating effectiveness of treatment.
Richard Sacra has been identified as having contracted the Ebola virus while working in Liberia. (Photo: University of Massachussetts Medical School, Handout/EPA)

American physician Richard Sacra, who contracted the disease in Liberia while caring for women in labor, has received an experimental drug called TKM-Ebola, made by Tekmira Pharmaceuticals Corp. TKM hasn't been approved yet, and the Food and Drug Administration has put its trial on a partial clinical hold while investigating side effects. But the agency allowed Sacra to receive it for compassionate use.
Repatriation and Treatment

- Convalescent plasma
- ZMapp
- TKM-Ebola
- Brincidofovir
- Favipiravir (T-705, Avigan)
- Real time monitoring and 24/7 supportive care in specialized medical centers
• Applies to the development or testing of drugs / biologicals to reduce or prevent serious, life-threatening conditions caused by exposure to lethal or permanently disabling toxic agents (chemical, biological, radiological, or nuclear substances), where human efficacy trials are not feasible or ethical. The animal rule was finalized by the FDA and authorized by the United States Congress in 2002, following the September 2011 attacks and concerns regarding bioterrorism.

• The FDA can rely on evidence from animal studies to provide substantial evidence of product effectiveness when:

1: There is a reasonably well-understood mechanism for the toxicity of the agent and its amelioration or prevention by the product;
2: The effect is demonstrated in either:
   2.1: More than one animal species expected to react with a response predictive for humans; or
   2.2: One well-characterized animal species model (adequately evaluated for its responsiveness in humans) for predicting the response in humans.
3: The animal study endpoint is clearly related to the desired benefit in humans; and
4: Data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information in animals or humans is sufficiently well understood to allow selection of an effective dose in animals, and it is therefore reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans.