Clinical Management of EBOLA VIRUS

September 30, 20XX

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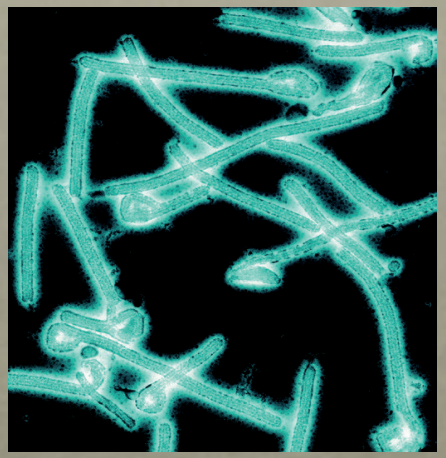


Figure 1 | The Ebola virus.

Geisbert TW. Ebola therapy protects severely ill monkeys. *Nature*. 2014 Aug 29.

Disclosure Statement

- I have no actual or potential conflict of interest in relation to this presentation.

Learning Objectives

- Assess a patient case and examine the appropriate precautions to use in the care of said patient
- Analyze literature associated with Ebola medication treatments currently in clinical trials
- Discriminate appropriate patient types for each available treatment

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.

Introduction

- 2014 outbreak of Ebola in West Africa
  - Largest in history
  - 4 U.S. citizens contracted the disease while in Africa
  - Successfully treated here in the U.S.
    - 2 received a monoclonal Ab treatment (ZMapp®)
    - 1 received a blood transfusion from an Ebola survivor + undisclosed drug
    - 1 received undisclosed drug
    - All received supportive care (fluids, electrolytes, etc.)

CNN. http://www.cnn.com/2014/08/04/health/experimental-ebola-serum/.

NBC News. http://www.nbcnews.com/storyline/ebola-virus-outbreak/new-ebola-patient-arrives-u-s-treatment-n199141.

FoxNews. http://www.foxnews.com/health/2014/09/09/ebola-infected-patient-to-be-brought-from-west-africa-to-atlanta-hospital/.

NBC News. http://www.nbcnews.com/storyline/ebola-virus-outbreak/exclusive-ebola-survivor-kent-brantly-donates-blood-treat-rick-sacra-n201131.

USA Today. http://www.usatoday.com/story/news/nation/2014/09/11/experimental-ebola-treatment/15443441/.

WEST AFRICA
Ebola Outbreak

1st Ebola outbreak in West Africa

4 countries:

- Guinea
- Sierra Leone
- Liberia
- Nigeria

Likely host = bats

Ebola is fatal in

55-60%

of cases reported in this outbreak.

Can be as high as 90%

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

http://www.cdc.gov/vhf/ebola/pdf/west-africa-outbreak-infographic.pdf

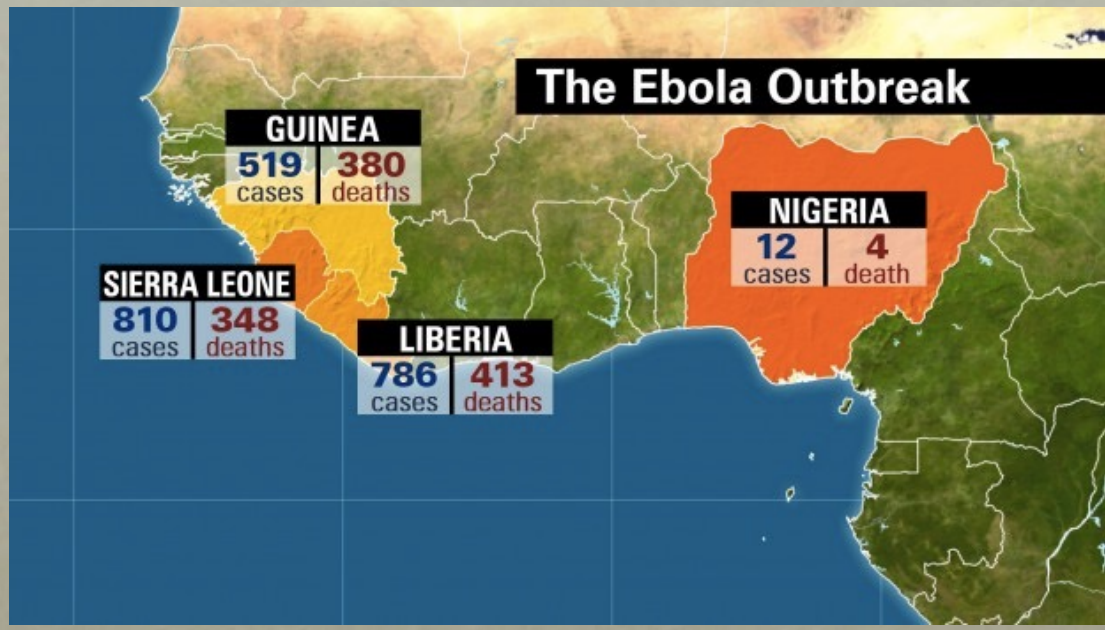
Introduction

- Increasing global nature of our travel
- Highly virulent pathogen
  - Causes severe hemorrhagic fever
  - Currently no cure
- Centers for Disease Control and Prevention (CDC)
  - Ebola is a **Category A** bioterrorism agent / disease
    - High mortality rate
    - Public panic / social disruption
    - Requires special action for public health preparedness

CDC. http://www.bt.cdc.gov/agent/agentlist-category.asp Accessed September 15, 2014

Epidemiology

- As of 13 September, 2014
  - 2453 deaths
  - 4963 cases (suspected, probable, or confirmed)

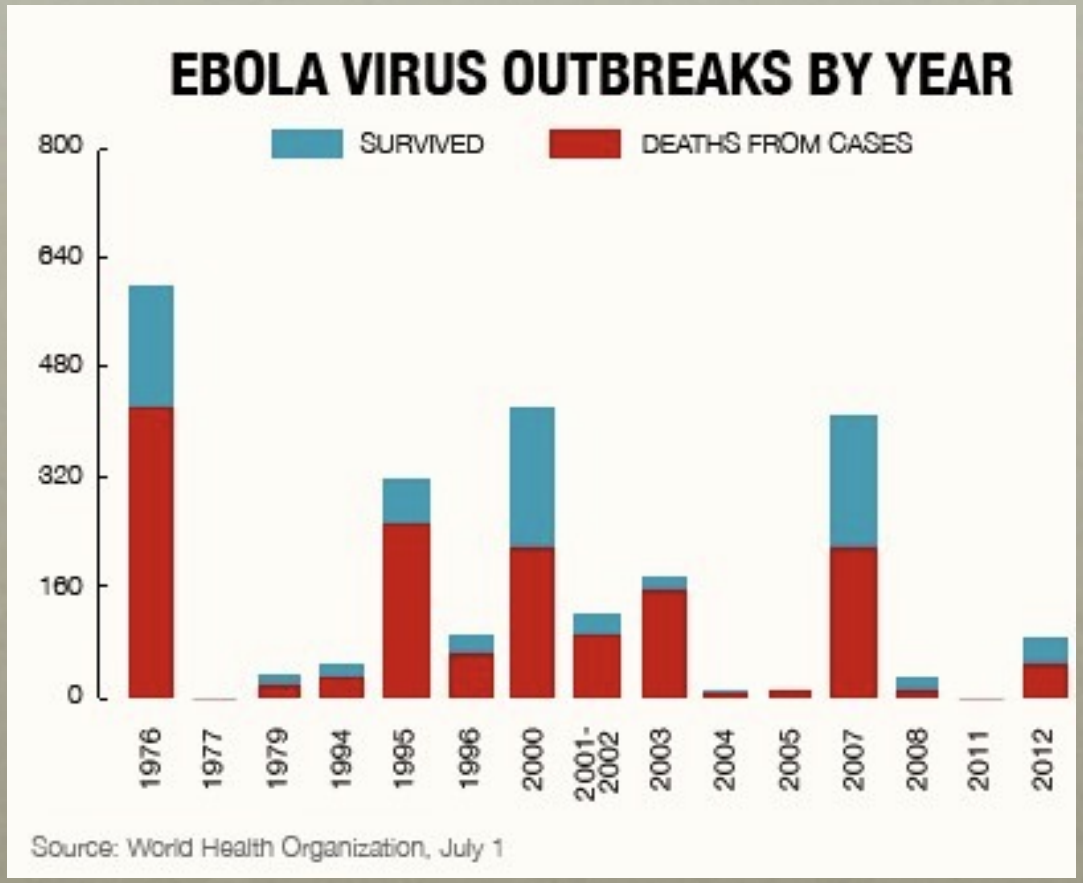


www.foxnews.com

World Health Organization. http://www.afro.who.int/en/clusters-a-programmes/dpc/epidemic-a-pandemic-alert-and-response/outbreak-news/4264-ebola-virus-disease-west-africa-28-august-2014.html Accessed September 8, 2014.

Epidemiology

- First Ebola virus species discovered in 1976
  - 2 simultaneous outbreaks
    - Democratic Republic of Congo, near the Ebola River
    - Sudan
- Since 1976, outbreaks have occurred sporadically



http://www.cnn.com/2014/07/25/health/ebola-outbreak-experimental-vaccines/

World Health Organization. http://www.who.int/mediacentre/factsheets/fs103/en/. Accessed September 8, 2014.

Country	Case definition	Cases			Deaths	
		Total	Past 21 days	Past 21 days/total cases (%)	Total	Deaths/total cases (%)
Guinea	Confirmed	743	276	37	429	58
	Probable	162	21	13	162	100
	Suspected	31	11	35	4	13
	All	**936**	**308**	**33**	**595**	**64**
Liberia	Confirmed	790	546	69	563	71
	Probable	1078	539	50	472	44
	Suspected	539	298	55	261	48
	All	2407	1383	57	1296	54
Sierra Leone	Confirmed	1464	583	40	514	35
	Probable	37	0	0	37	100
	Suspected	119	70	59	11	9
	All	**1620**	**653**	**40**	**562**	**35**
Total		**4963**	**2344**	**47**	**2453**	**49**

World Health Organization. http://www.afro.who.int/en/clusters-a-programmes/dpc/epidemic-a-pandemic-alert-and-response/outbreak-news/4264-ebola-virus-disease-west-africa-28-august-2014.html Accessed September 8, 2014.

Etiology

Filoviridae family of viruses

5 types

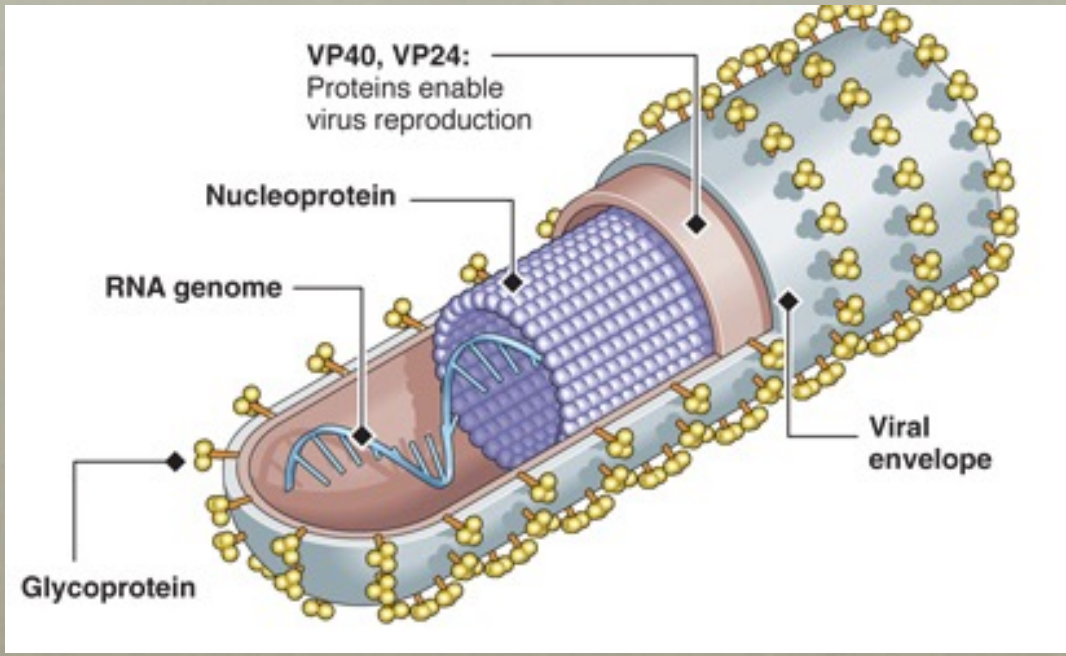
- Zaire (ZEBOV)
- Sudan (SUDV)
- Tai Forest (TAFV) or Cote d'Ivoire (CIEBOV)
- Bundibugyo (BDBV) – newest strain (2007)
- Reston (RESTV)

http://sweetclipart.com/cute-monkey-banana-635

CDC. http://www.cdc.gov/vhf/virus-families/filoviridae.html Accessed September 15, 2014

Etiology

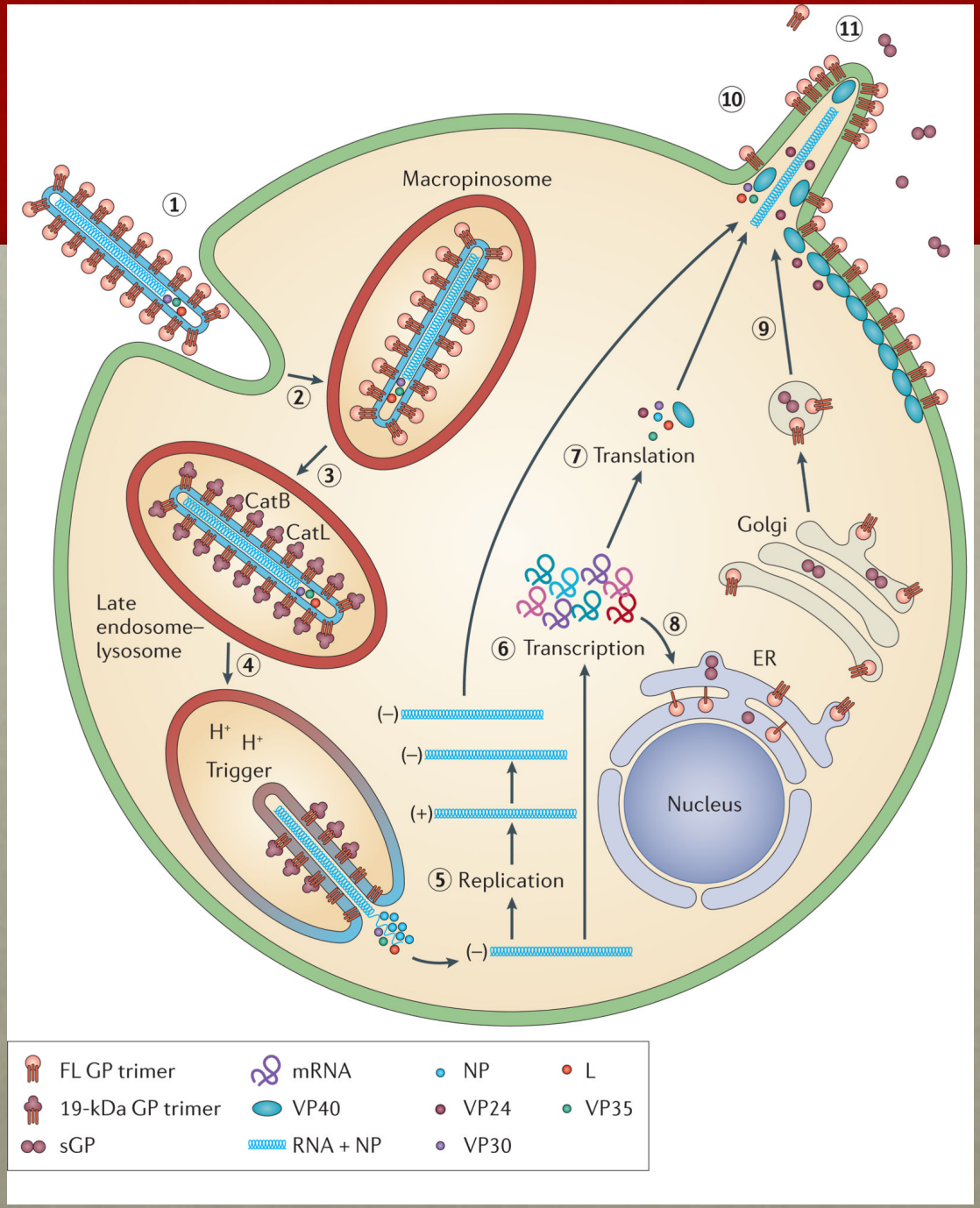
- RNA virus
  - Single-stranded
  - Negative-sense
  - Enveloped
    - Easier kill w/ surface disinfectants
  - GP (glycoprotein) spikes
    - Allow binding host cells
    - The only protein on the surface of the virus → therapeutic target
  - sGP (secretory glycoprotein) bind circulating neutralizing antibodies in host



St. Catherine's Standard.
http://www.stcatharinesstandard.ca/2014/07/30/what-you-need-to-know-about-ebola

Etiology

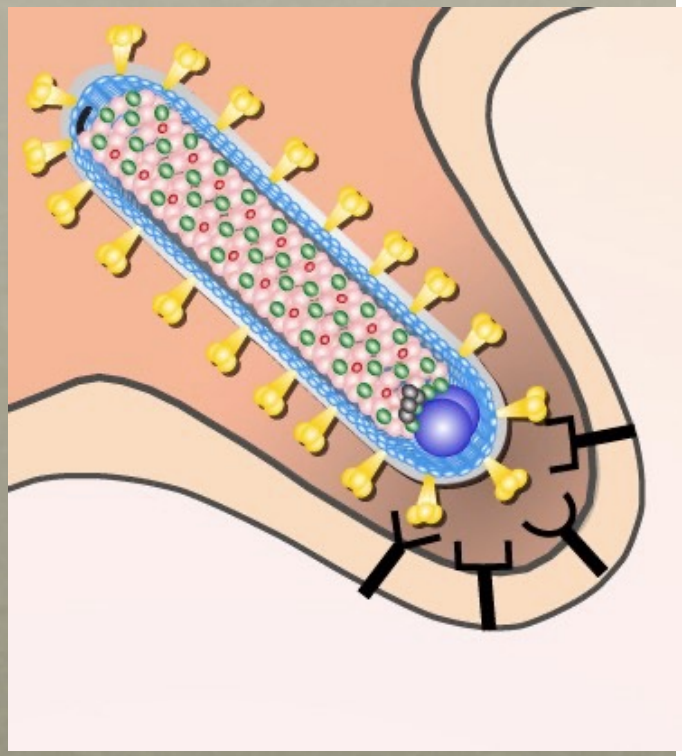
- Replicates entirely in the cytoplasm
  - Negative-sense strand of RNA makes positive-sense RNA (=mRNA) first
  - Positive-sense (mRNA) → proteins

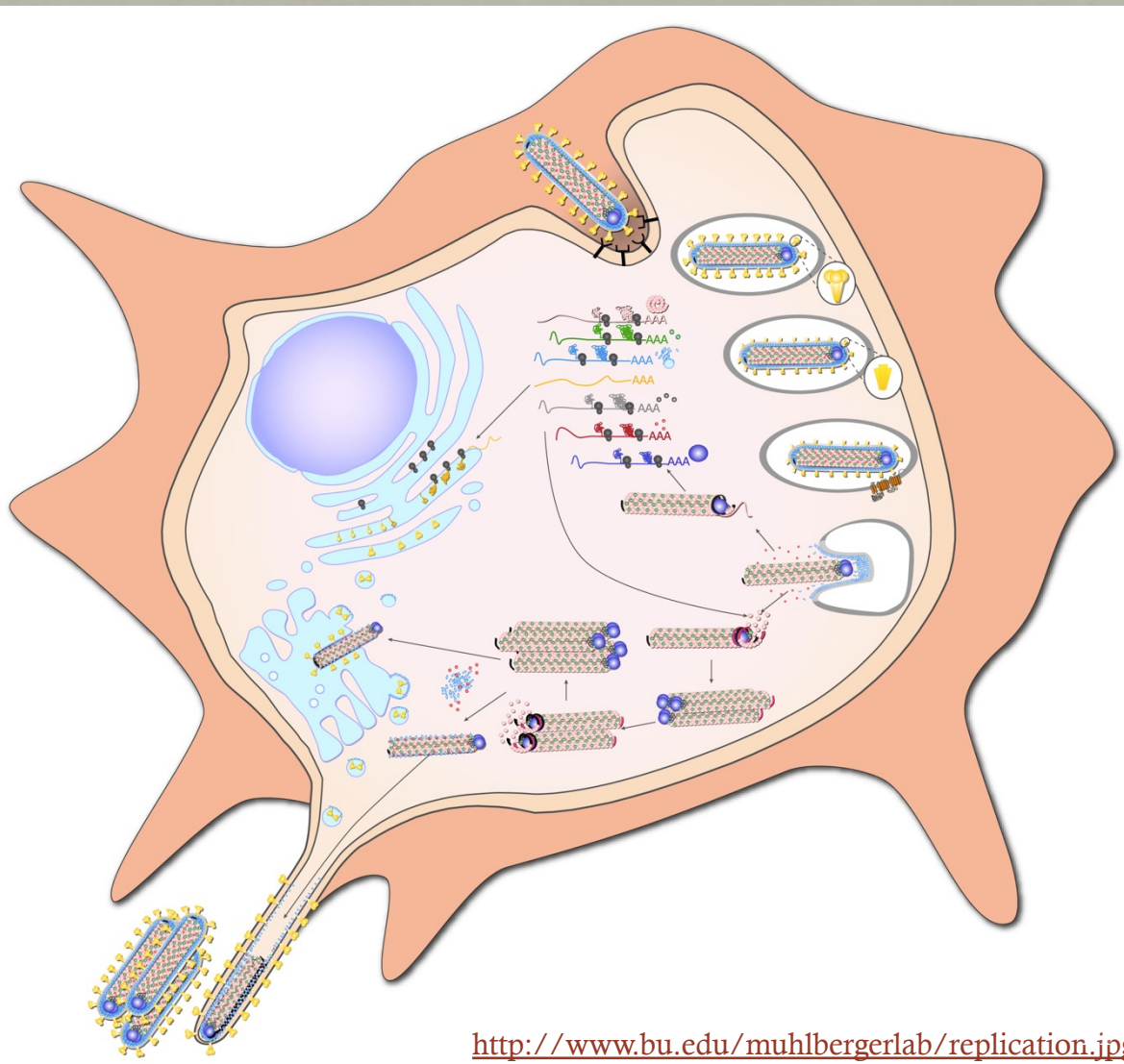


White JM, Schornberg KL. A new player in the puzzle of filovirus entry. *Nat Rev Microviol*. 2013; 10(5): 317-322.

Etiology

- Glycoprotein Spikes allow virus to enter host cell

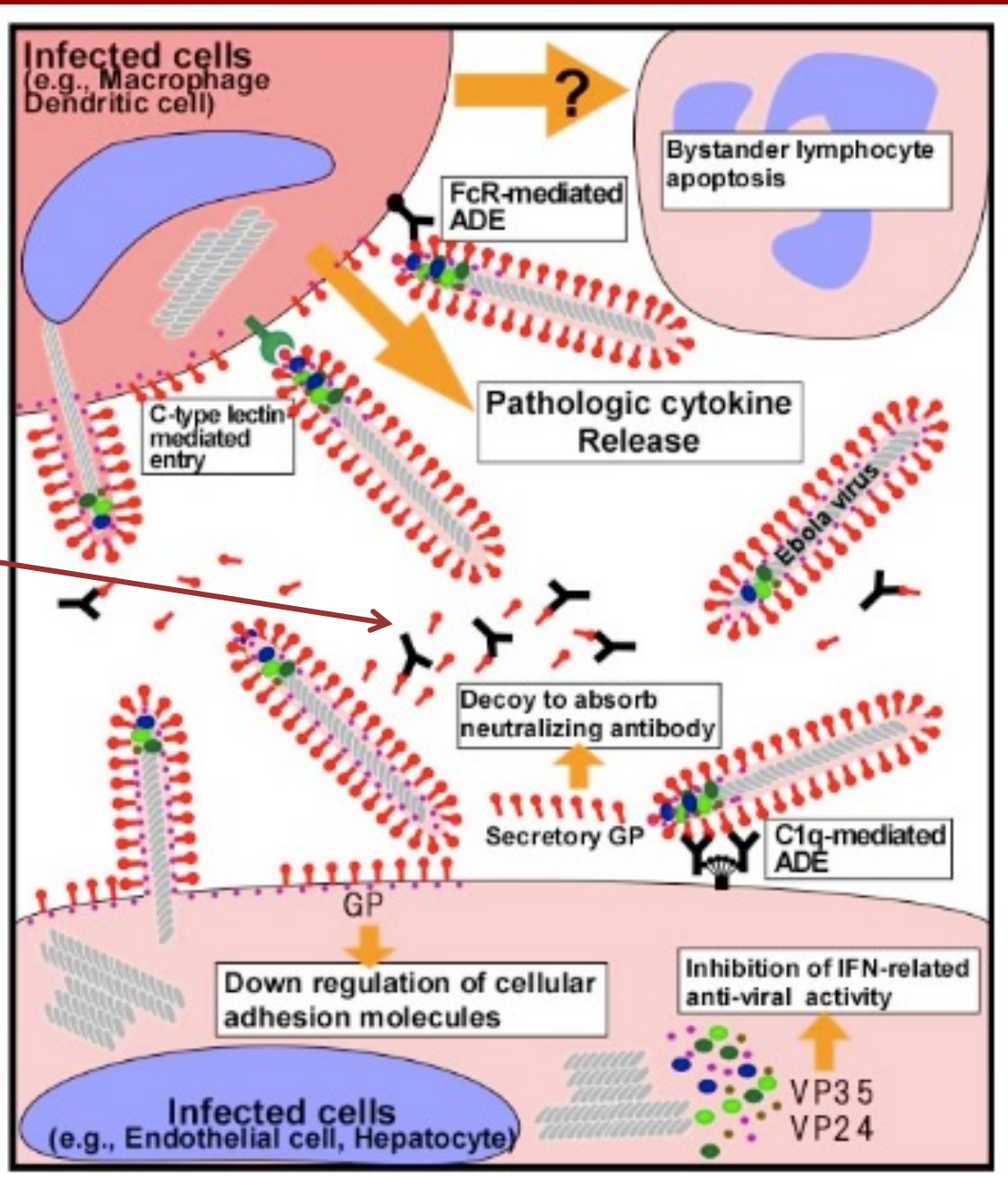




http://www.bu.edu/muhlbergerlab/replication.jpg

Etiology

- Secretory GP neutralizes any host Ab's



Ebola virus pathogenesis and host immunity

http://www.czc.hokudai.ac.jp/epidemiol/research/ebola3e_L.png

Family	Representative Viruses	Type
Picornaviridae	Poliovirus, Coxsackievirus, Enterovirus, Rhinovirus, Hep A	(+) RNA
Togaviridae	Rubella virus, Equine encephalitis viruses	
Flaviviridae	Yellow fever virus, Dengue virus, West Nile virus, Hep C	
Coronaviridae	Coronaviruses (inclu. SARS)	
Rhabdoviridae	Rabies virus, Vesicular stomatitis virus	(-) RNA
Filoviridae	Marburg virus, **Ebola virus**	
Paramyxoviridae	Parainfluenza virus, RSV, Mumps virus, Rubeola (measles) virus	
Orthomyxoviridae	Influenza A, B, C viruses	
Arenaviridae	Lassa fever virus	
Reoviridae	Rotavirus	dsRNA
Retroviridae	Lentivirus (HIV)	dsRNA
Hepadnaviridae	Hep B	DNA
Parvoviridae	Parvovirus	
Papovaviridae	HPV	
Adenoviridae	Adenovirus	
Herpesviridae	Herpes simplex virus, Varicella-zoster virus, Herpes-zoster virus, Epstein-Barr virus, Cytomegalovirus	
Poxviridae	Variola (smallpox)	

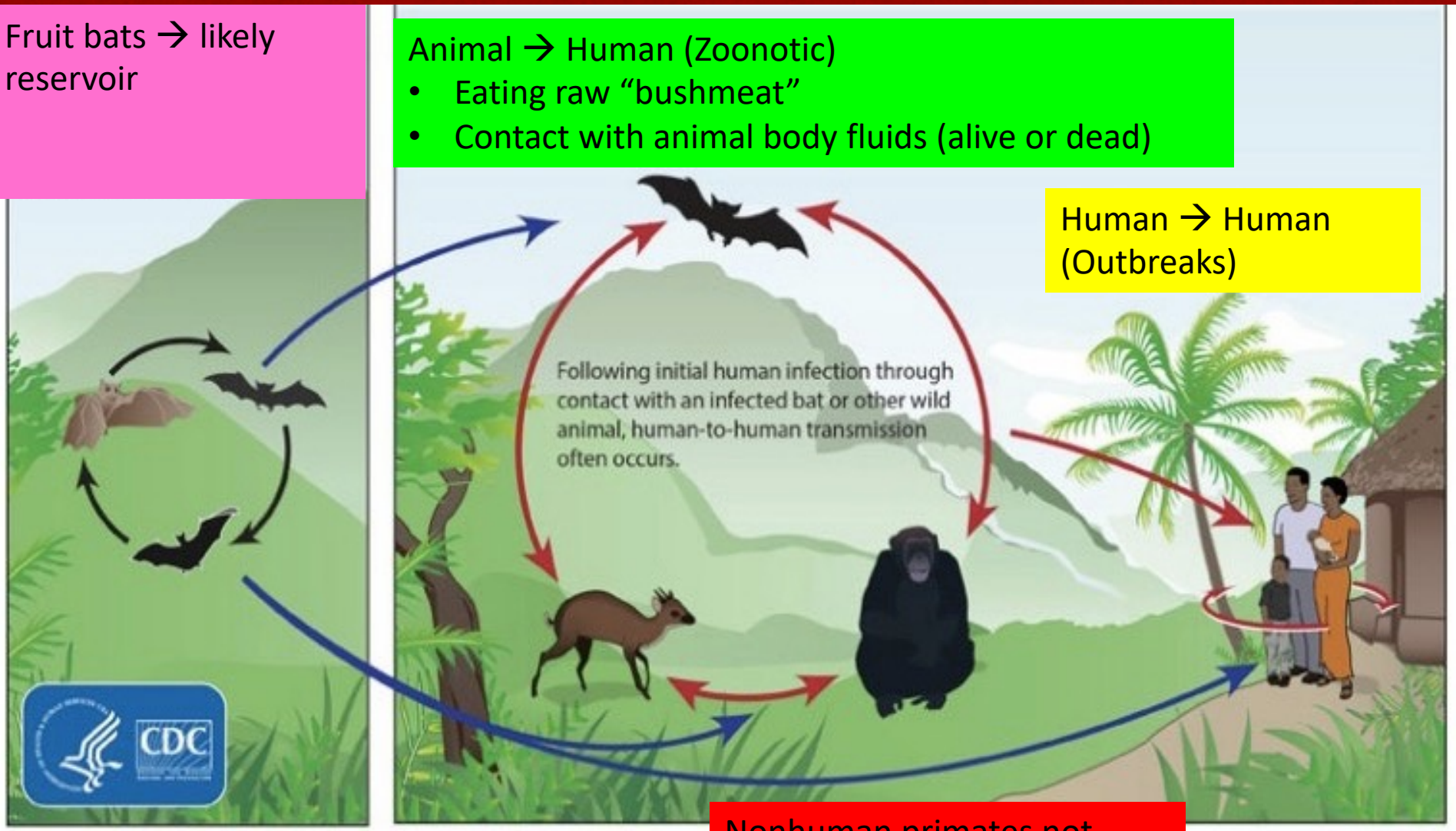
Transmission

Fruit bats → likely reservoir

Animal → Human (Zoonotic)

- Eating raw "bushmeat"
- Contact with animal body fluids (alive or dead)

Human → Human (Outbreaks)



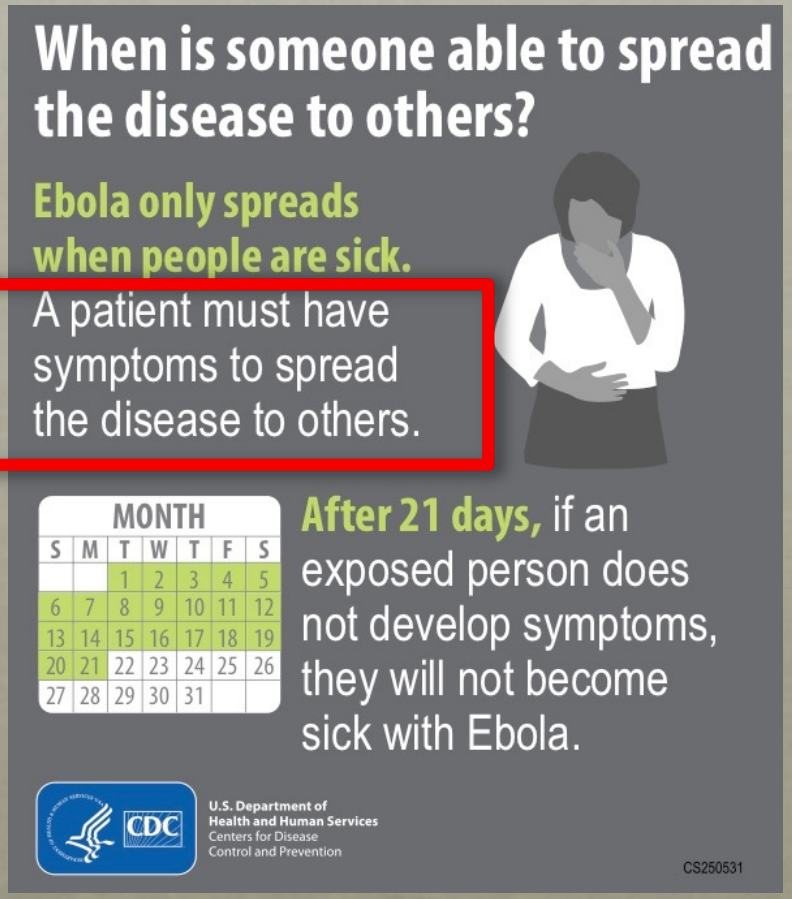
Nonhuman primates not likely reservoir; unfortunate host like humans

World Health Organization. http://www.who.int/mediacentre/factsheets/fs103/en/.

CDC. http://www.cdc.gov/vhf/ebola/resources/virus-ecology.html.

Transmission

- Body secretions of infected person ***who is symptomatic***
  - Blood
  - Vomit
  - Feces
  - Urine
  - Saliva
  - Sweat
  - Semen (virus can remain up to 3 months after clinical recovery)
- Direct contact with
  - Mucosal surfaces
  - Skin abrasions (non-intact epithelium)
  - Contaminated needles



CDC. http://www.cdc.gov/vhf/ebola/pdf/west-africa-outbreak-infographic.pdf Accessed Sept 8, 2014

Facts *about* Ebola

You can't get Ebola through air

You can't get Ebola through water

You can't get Ebola through food

You can only get Ebola from touching bodily fluids of a person who is sick with or has died from Ebola, or from exposure to contaminated objects, such as needles. **Ebola poses no significant risk in the United States.**

Unlikely during outbreaks, but airborne transmission not an impossibility (see studies by Reed and Weingartl)

Exception: eating raw "bushmeat"

CDC. http://www.cdc.gov/vhf/ebola/pdf/infographic.pdf. Accessed September 8, 2014.

Transmission

http://tbandleprosy.weebly.com/transmission-and-treatment.html

- *May* be able to transmit via aerosolized particles
  - Biological warfare
  - Aerosolized ZEBOV infected Non-Human Primates
    - Clinical signs & time to death = parenteral inoculation
  - Pigs transmitted ZEBOV to NHPs via aerosols
    - ZEBOV causes a respiratory tract infection in pigs (not a hemorrhagic fever)
  - Epidemiological studies
    - Not a prominent method of transmission during outbreaks
    - Inoculating dose may be important

Weingartl HM, Embury-Hyatt-C, Nfon C, et al. Transmission of Ebola virus from pigs to non-human primates. *Scientific Reports*. 2012; 2 (811): 1-4.

Reed DS, Lackemeyer MG, Garza NL, et al. Aerosol exposure to Zaire Ebolavirus in three nonhuman primate species: differences in disease course and clinical pathology. *Microbes and Infection*. 2011; 13: 930-936.

Transmission

Stopping the **Ebola Outbreak**

Prevent Healthcare infection control, safe burial practices, avoiding bushmeat

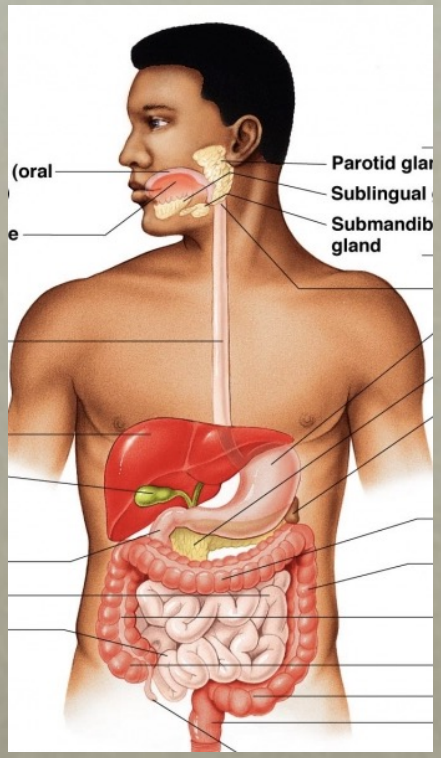
INFECTION CONTROL

SAFE BURIAL PRACTICES

http://www.cdc.gov/vhf/ebola/pdf/ghs-ebola-materials.pdf

Pathogenesis

- Ebola virus infects many cells / organs
  - Immune cells (monocytes, macrophages, dendritic cells)
    - Primary Targets for Ebola virus replication
  - Hepatocytes
  - Adrenal cortical cells
  - Spleen
  - Pancreas
  - Endothelial cells
  - Epithelial cells
  - Fibroblasts



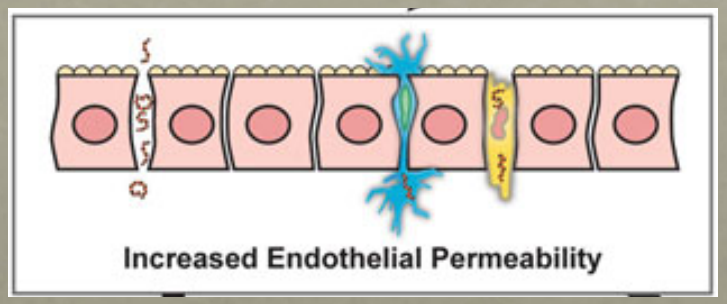
http://www.universityherald.com/

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

CDC. http://www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html. Accessed September 17, 2014.

Pathogenesis

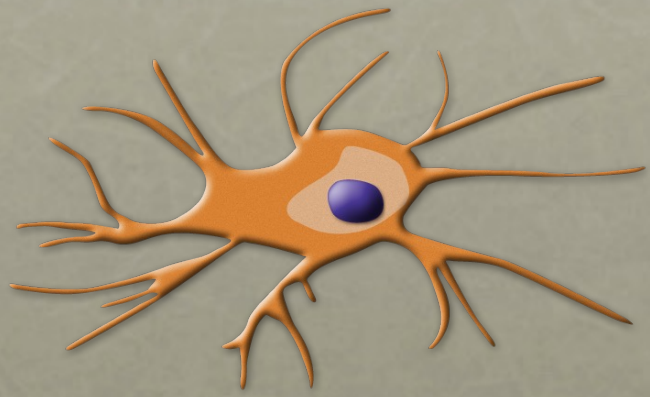
- Infected Monocytes / Macrophages
  - "Cytokine Storm" → Massive release of pro-inflammatory cytokines
    - IL-1Beta, 2, 6, 8, 10
    - TNF-alpha
    - Recruitment of additional immune cells
      - Increases # of hosts for virus to replicate
    - Vascular Leak (increased endothelial permeability)
      - Rapid dissemination of infected immune cells
      - Hemorrhage / Rash
    - Multi-organ failure and shock



Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Pathogenesis

- Infected Dendritic Cells (DCs)
  - Ebola VP24, VP35 play pivotal role
    - Block Type I Interferon anti-viral response
    - DCs unable to stimulate lymphocytes (T-cells)
    - Massive lymphocyte apoptosis
  - Unable to express co-stimulatory molecules that would illicit an immune response



http://www.nephronpower.com/2011/07/topic-discussion-dendritic-cells-and.html

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Pathogenesis

- Infected hepatocytes
  - Hepatocellular Necrosis
    - Dysregulation of clotting factors
      - Hemorrhage
      - Disseminated Intravascular Coagulopathy (DIC)
- Infected adrenal cortical cells
  - Adrenocortical necrosis
    - Impaired steroid synthesis
      - Hypotension

http://www.clipartof.com/gallery/clipart/kidney.html

CDC. http://www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html. Accessed September 17, 2014.

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Pathogenesis

- Laboratory Findings
  - Leukopenia
  - Elevated neutrophils
    - Left Shift
  - Thrombocytopenia
  - Elevated Amylase (pancreatic inflammation)
  - Elevated AST / ALT
  - Proteinuria
  - PTT prolongation / elevated INR

CDC. http://www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html. Accessed September 17, 2014.

Signs & Symptoms

Early Symptoms:

Ebola can only be spread to others after symptoms begin. Symptoms can appear from 2 to 21 days after exposure.

- Fever
- Headache
- Diarrhea
- Vomiting
- Stomach pain
- Unexplained bleeding or bruising
- Muscle pain

Incubation Period:
2-21 Days
(Mean = 8-10 days)

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CDC. http://www.cdc.gov/vhf/ebola/pdf/west-africa-outbreak-infographic.pdf.

Signs & Symptoms

Prolonged convalescence if survival

Abrupt Onset
8-10 days after exposure
(Range: 2-21 days)

If patient survives, usually starts improving after 6-11 days of symptoms

Initial (5 days)
- Fever
- Chills
- Myalgia
- Malaise

Progression to
- N/V/D
- (Diarrhea can be severe and watery)
- Abdominal Pain
- Conjunctival Injection
- SOB, Chest Pain
- Cerebral Edema
- Confusion
- Seizures

Hemorrhagic Manifestations
- Frank hemorrhage less common
- Petechiae
- Ecchymosis/Bruising
- Oozing from venipuncture sites
- Mucosal hemorrhage
- Thrombocytopenia
- Spontaneous Miscarriage

Multi-Organ Failure / Septic Shock

DEATH

Day 5-7 after exposure: May develop diffuse erythematous maculopapular rash
(face, neck, trunk, and arms)
Can desquamate

If death occurs, usually happens after 6-16 days of symptoms

CDC. http://www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html.

Signs & Symptoms

- Internal or External Bleeding:
  - Rashes
  - Bleeding gums
  - Conjunctival injection (“red eye”)
  - Epistaxis (nose bleeds)
  - Hematemesis (blood in vomit)
  - Hemoptysis (blood in sputum)
  - Melena (dark blood in stool)
  - Hematochezia (bright red blood in stool)
  - Hematuria (blood in urine)

http://umms.org/shore-health/health/medical/ency/images/ebola-virus

CDC. http://www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html.

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- What s/s could suggest EBOV disease?

Differential Dx

- Nonspecific symptoms early in the course
- Must r/o more common infectious diseases:
  - Malaria
    - Travel to sub-Saharan Africa or other tropical areas
  - Dengue
    - Travel to Latin American or Asia
  - Typhoid Fever
  - Meningococcal Disease
  - PNA
  - Other bacterial infections

CDC. Fever in returned travelers. http://wwwnc.cdc.gov/travel/yellowbook/2014/chapter-5-post-travel-evaluation/fever-in-returned-travelers. Accessed September 17, 2014.

Definitions: World Health Organization

Classification	Criteria	
Suspect Case (Alert Case)	Sudden onset of high fever (>38°C) AND • Contact with a suspected, probable, or confirmed Ebola case • OR • Contact with a dead or sick animal	
	Any person with sudden onset of high fever and at least 3 of the following: • Headache • Abdominal Pain • Vomiting • Diarrhea • Anorexia	• Lethargy • Aching muscles / joints • Difficulty swallowing • Breathing difficulties • Hiccup
	Any person with unexplained bleeding: • Bloody diarrhea • Blood in urine • Blood in vomit • Blood in sputum (hemoptysis)	• Bleeding gums • Bleeding into skin (purpura) • Bleeding into eyes • Nose bleeds (epistaxis)
	Any sudden, unexplained death	

World Health Organization. http://www.who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf?ua=1

Definitions: Centers for Disease Control and Prevention

Classification	Criteria
"Person Under Investigation"	Clinical Criteria (Fever + additional symptoms) AND Epidemiologic Risk Factors w/in Past 3 weeks (21 days) prior to onset of symptoms: • Contact w/ blood or body fluids of patient with known or suspected EBOV • Residence in or travel to an area where EBOV transmission is active • Participation in a funeral / burial rituals in an endemic area • Direct handling of bats or NHPs from endemic area

CDC. Case Definition for Ebola Virus Disease. http://www.cdc.gov/vhf/ebola/hcp/case-definition.html.

Definitions: World Health Organization

Classification	Criteria
Probable Case	A suspect case known to have had contact with a suspect, probable, or confirmed case
	A suspect case **who died** and had an epidemiological link to a confirmed case but who never had laboratory confirmation of the disease
Confirmed Case	Person tests positive for Ebola virus antigen (laboratory confirmation) • IgM Ab OR • RT PCR (detects viral RNA)
Non-Case	Any suspected or probable case with a negative lab result

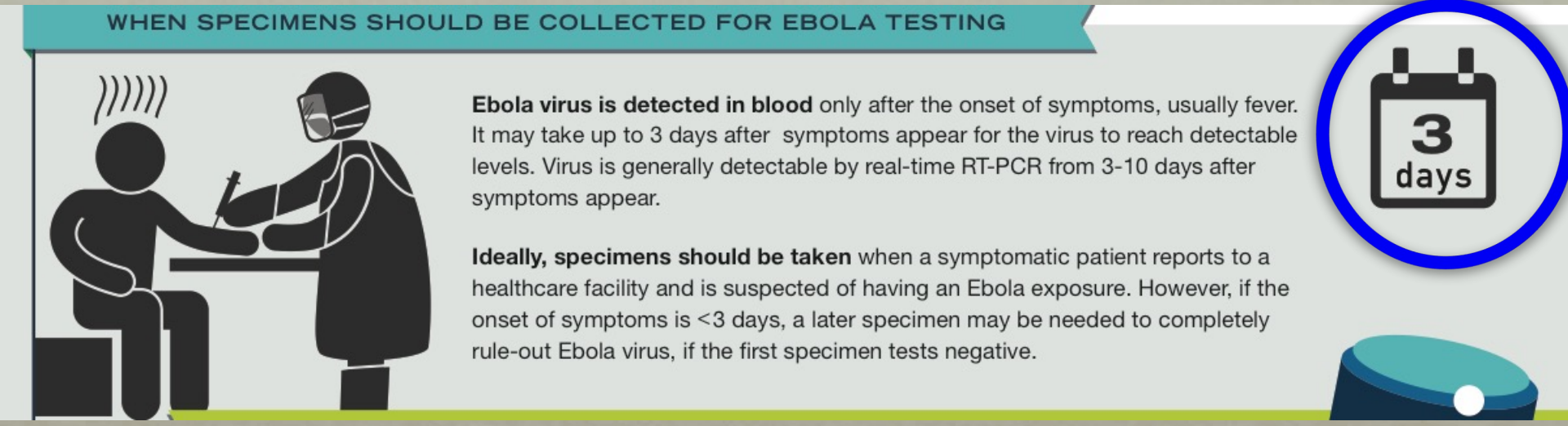
RT PCR, reverse transcriptase polymerase chain rxn

World Health Organization. http://www.who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf?ua=1

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- Upon further questioning, ZB confirms that he had taken care of several patients with the same symptoms while in Africa.
- How would you classify ZB's case according to the World Health Organization criteria?

Diagnostic Testing

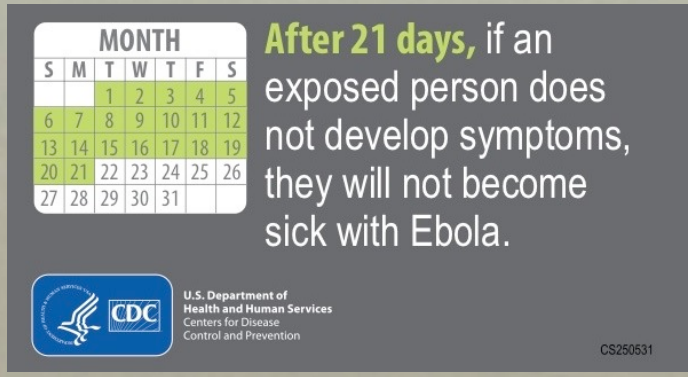


- Samples taken from suspected human and animal Ebola cases are an “extreme biohazard risk.”

World Health Organization. http://www.who.int/mediacentre/factsheets/fs103/en/.
CDC. http://www.cdc.gov/vhf/ebola/pdf/ebola-lab-guidance.pdf.

Precautionary Measures

- Quarantine / trace-back investigations
  - Very effective to prevent spread
- All contacts of suspected cases
  - "Conditional Release & Controlled Mvmt"
    - Surveillance – self-monitor for fever BID
    - X 21 days
  - Must notify public health authority of:
    - s/s
    - Intended travel



IDSA Guidelines. http://www.idsociety.org/HAN_20140803/

CDC. http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html.

CDC. http://www.cdc.gov/vhf/ebola/hcp/monitoring-and-movement-of-persons-with-exposure.html.

Definitions: World Health Organization

Classification	Criteria
Case Contacts	Any person having been exposed to a suspect, probable, or confirmed case of Ebola in at least 1 of the following ways: • Slept in same household with the case • Direct physical contact with the case (alive or dead) during illness • Direct physical contact with the dead case at the funeral • Touched blood / body fluids of case during the illness • Touched clothes / linens of case during the illness • Been breastfed by the case (baby)
Dead / Sick Animal Contacts	Any person having been exposed to a dead or sick animal in at least 1 of the following ways: • Direct physical contact with the animal • Direct physical contact with the animal's blood / body fluids • Carved up the animal • Eaten raw "bushmeat" (ape, bat)
Laboratory Contacts	Any person having been exposed to biological material in at least one of the following ways: • Direct contact with specimens collected from suspected cases (animal or human)

World Health Organization. http://www.who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf?ua=1

Precautionary Measures

- Disinfecting Surfaces
  - 10% hypochlorite (bleach) soln
  - Quaternary ammonium products
  - Phenolic products

IDSA Guidelines. http://www.idsociety.org/HAN_20140803/

CDC. http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html.

CDC. http://www.cdc.gov/vhf/ebola/hcp/monitoring-and-movement-of-persons-with-exposure.html.

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- ZB's wife has been taking care of him for the past 5 days, including cleaning the toilet where ZB has had episodes of emesis and diarrhea and cleaning his bed linens.
- How should ZB's wife be categorized?
- What precautionary measures should be taken for her?

[Hospital X] Policies

- Summary of [Hospital X] Policies that would apply to suspect, probable, or confirmed cases of EBOV:
  - Standard Precautions
  - Contact Precautions
  - Airborne Infection Precautions
  - OSHA Bloodborne Pathogen Standard

Infection	Transmission Based Precaution @ [Hospital X]	Duration
Ebola Virus	Contact	Duration of Illness
Hemorrhagic Fever – with history of travel to Africa (Notify Infection Prevention)	Airborne Infection Contact	Duration of Illness

[Hospital X] [Hospital System]. Transmission Based Precautions Reference Table. Revised

[Hospital X] Policies

- **Standard Precautions**
  - Hand Hygiene
    - Hand washing
      - Soap and H20 x15 seconds
      - Following patient contact / after removal of PPE
  - PPE (=Personal Protective Equipment)
    - Gowns, Gloves, Face Shields, Masks, Eye Protection
      - During aerosol-generating procedures
      - During likely encounters with blood / body fluids (esp. sprays or splashes)

[Hospital X] [Hospital System]. Standard Precautions Infection Prevention. Approval Date: 10/11/2012.

[Hospital X] Policies

- **Contact Precautions**
  - Gloves and Gowns
    - ***For all contact*** w/ patient and patient's environment
  - Dedicate supplies and equipment to patient
    - Stethoscopes, pulse oximetry, other durable medical equipment

[Hospital X] Policies

- **Airborne Precautions**
  - Negative Pressure Room
  - Portable HEPA unit turned to highest setting
    - Exceptions: ICU 20, CTRU 1, and ED Rm 31
  - Door must remain closed
  - Respirators (NIOSH approved) – N95
    - Fit testing required
  - Surgical Face Mask
    - Patient must wear during transport w/in hospital
    - Health care workers transporting patient need not wear respirators as long as pt is masked

CDC Statement

- *"Any U.S. hospital that is following CDC's infection control recommendations and can isolate a patient in a private room is capable of safely managing a patient with Ebola Virus Disease. CDC recommends that U.S. hospitals implement standard, contact, and droplet precautions."*

Centers for Disease Control and Prevention. Safe management of patients with EVD in US hospitals. http://www.cdc.gov/vhf/ebola/hcp/patient-management-us-hospitals.html. Accessed September 17, 2014.

[Hospital X] Policies vs CDC

- CDC also recommends:
  - Restrict visitors and keep a logbook of all persons entering the room
  - Wearing eye protection (goggles or face shield) during all interactions
    - Eyes are a mucous membrane
    - This is included in [Hospital X]'s "***Special*** Airborne/Contact Precautions" policy
      - Ebola currently listed as Airborne/Contact

Centers for Disease Control and Prevention. Safe management of patients with EVD in US hospitals. http://www.cdc.gov/vhf/ebola/hcp/patient-management-us-hospitals.html. Accessed September 17, 2014.

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- You are the pharmacist working in the ED when ZB presents. The ED attending physician asks you if [Hospital X] has hospital isolation and / or transmission prevention policies for managing suspect or probable cases of Ebola. What do you say?

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- ZB's wife is worried that he may have transmitted the disease to other passengers during his flight back from Africa. How would you respond to this concern?

Treatment

- Supportive Care

Symptom	Medication	Notes
Fever	APAP	Avoid NSAIDs (due to bleeding risk)
Pain	APAP or Morphine	Avoid NSAIDs
Dehydration	Fluids, Electrolyte Repletion	
N/V/D	Anit-emetics	May facilitate rehydration
Hemorrhage	Blood Transfusion	
Hypoxia	Oxygen	
Shock	Vasopressors to maintain BP Broad-spectrum Antbx if 2^{o} likely	

World Health Organization. Clinical management of patients with viral hemorrhagic fever. 30 March 2014. http://www.who.int/csr/resources/publications/clinical-management-patients/en/. Accessed September 16, 2014.

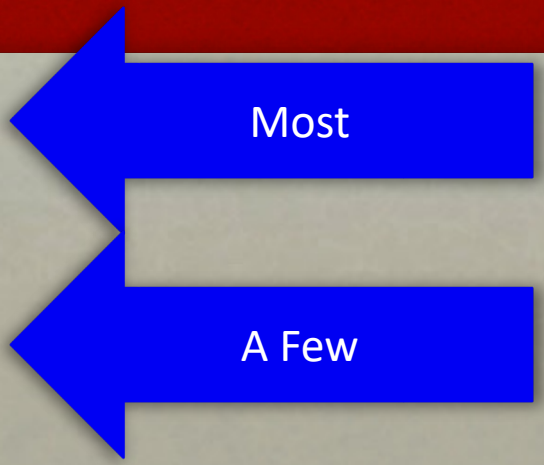
Centers for Disease Control and Prevention. Ebola virus disease information for clinicians in U.S.healthcare settings. http://www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html. Accessed September 17, 2014.

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- The ED attending physician asks you if it would be OK to give ZB a dose of ibuprofen 800mg to help manage his muscle aches. What do you say?

Treatment

- Pre-Clinical
  - Animal studies / Proof-of-Concept Lab Studies
- Phase I
  - Small group of humans
  - Safety
- Phase II
  - Large group of humans
  - Efficacy, further evaluation of safety
- Phase III
  - Larger group of humans
  - Confirm effectiveness / safety, compare to commonly used treatments
- FDA Approval
- Phase IV
  - Post-FDA approval
  - Long-term safety & efficacy, effect in many different populations



Currently no approved vaccines or treatments for Ebola

http://www.nlm.nih.gov/services/ctphases.html.

Treatment

- FDA Compassionate Use Regulation
  - AKA “Expanded Access”
  - “Use of an investigational drug outside of a clinical trial to treat a patient with a serious or immediately life-threatening disease or condition who has no comparable or satisfactory alternative treatment options.”
  - =Not FDA-approved for safety and efficacy
  - Channel through which U.S. citizens received treatment

FDA. http://www.fda.gov/forconsumers/byaudience/forpatientadvocates/accesstoinvestigationaldrugs/ucm176098.htm.

Treatment Modalities Under Investigation

Vaccination	Passive Immunotherapy	Viral RNA Interference	Inhibition of other viral processes	Symptomatic Treatment
EBOV genetic material or surface glycoproteins	Blood Transfusions from Survivors	Small interfering RNAs (siRNA)	Viral entry inhibitors	Protein C
	Monoclonal Antibodies	Complimentary RNA base sequences that bind EBOV RNA (PMO's)	Viral release inhibitors	Interferon Beta Therapy
			Protease inhibitors	

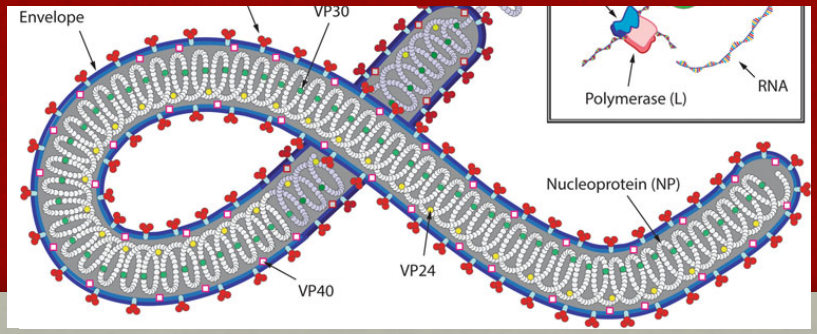
Treatment Modalities Under Investigation

Mostly Pre-exposure

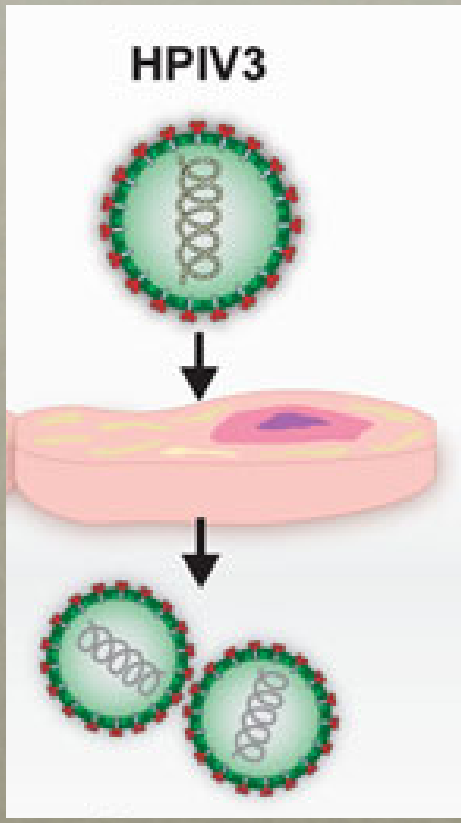
Mostly Post-exposure / Tx

Vaccination	Passive Immunotherapy	Viral RNA Interference	Inhibition of other viral processes	Symptomatic Treatment
EBOV genetic material or surface glycoproteins	Blood Transfusions from Survivors	Small interfering RNAs (siRNA)	Viral entry inhibitors	Protein C
	Monoclonal Antibodies	Complimentary RNA base sequences that bind EBOV RNA (PMO's)	Viral release inhibitors	Interferon Beta Therapy
			Protease inhibitors	

Vaccines



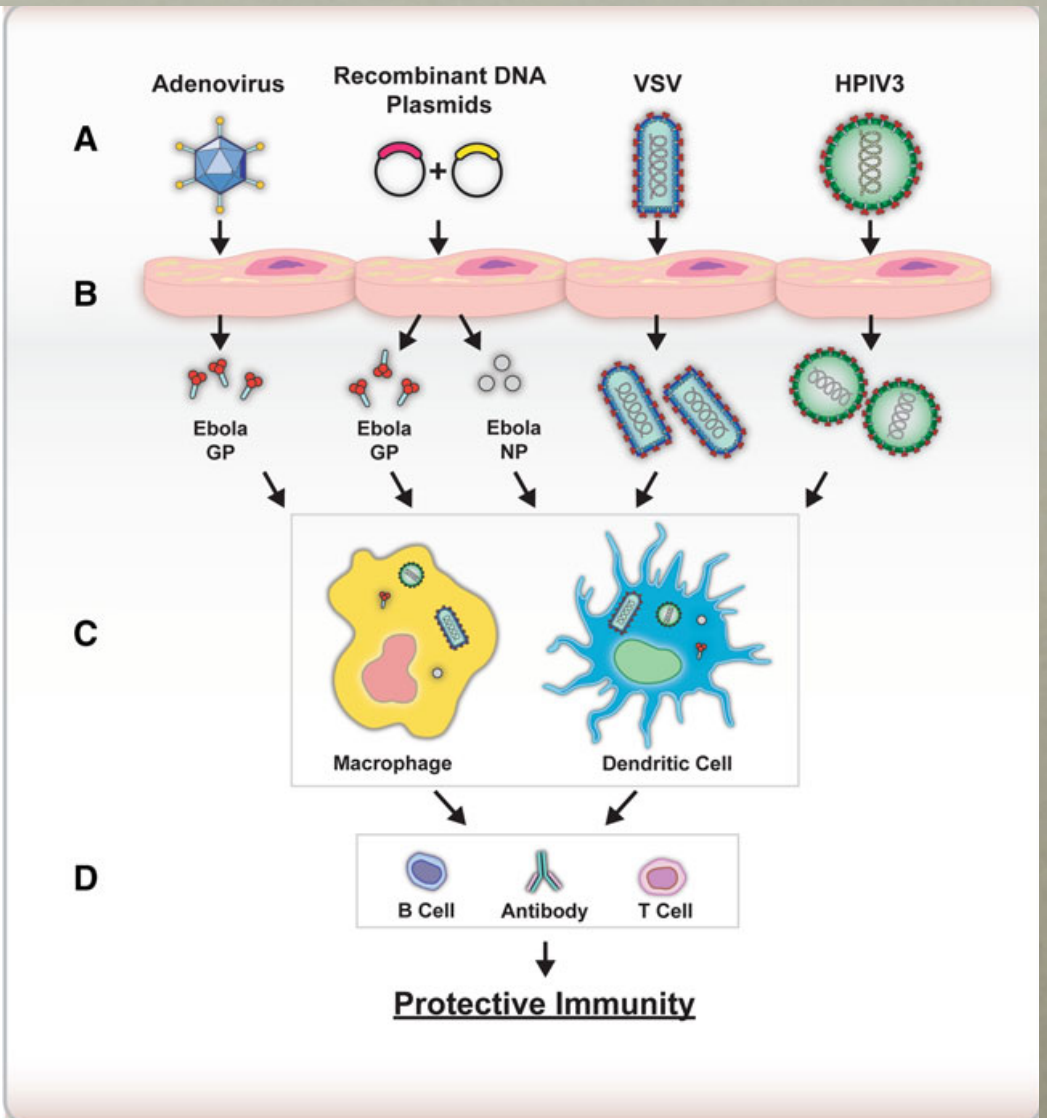
- Inactivated whole Ebola virions
  - First Ebola vaccine developed
  - Ineffective in rodents
- Using other viruses as vectors / carriers
  - Carries EBOV genetic material into cell
  - Cell makes proteins or replicates the virus vector
  - Immune system generates antibodies
  - Effective in animal models



Chupurnov AA, Chernukhin IV, Ternovoi VA, et al. Attempts to develop a vaccine against Ebola fever. Vopr Virusol. 1995; 40(6): 257-60.

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Vaccines



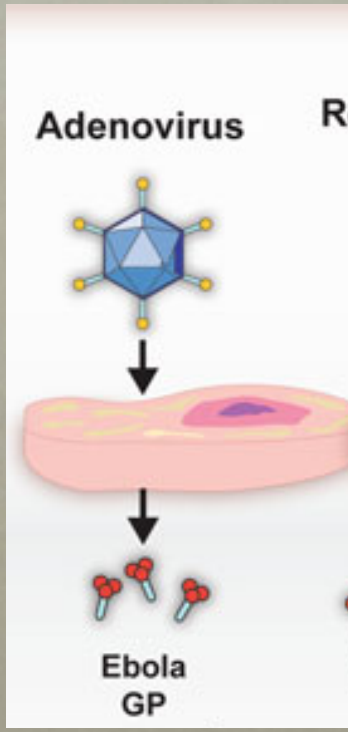
- Non-replicating viral vectors:
  - Adenovirus
- DNA Plasmids
- Live Attenuated (replicating) viral vectors:
  - Vesicular Stomatitis Virus (VSV)
  - Human Parainfluenza Virus 3 (HPIV3)

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Vaccines

Vaccine Vector	EBOV genetic material it carries	How vaccine given	Subjects	Study Results	Authors (Date)
Adenovirus	GP + NP	X1 dose 4 weeks before ZEBOV exposure	NHPs	4/4 protected	Sullivan et al (2003)
Adenovirus enhanced w/ CMV promoter	GP	X1 dose 30 mins AFTER ZEBOV exposure	Mice	100% protected	Richardson et al (2009)

GP, glycoprotein; NP, nucleoprotein; NHP, non-human primates; CMV, cytomegalovirus



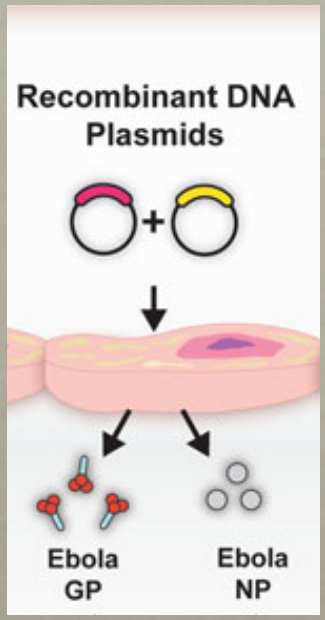
Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus hemorrhagic fever in non-human primates. *Nature*. 2003; 424: 681-684.

Richardson JS, Yao MK, Tran KN, et al. Enhanced protection against Ebola virus mediated by an improved adenovirus-based vacine. PLoS ONE. 2009; 4(4): e5308.

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Vaccines

Vaccine Vector	EBOV genetic material it carries	How vaccine given	Subjects	Study Results	Authors (Date)
DNA Plasmid	GP	1 inject q2wks x 3 doses 3 months before ZEBOV exposure	Guinea Pigs	4/4 protected	Sullivan et al (2000)
	GP + NP			4/4 protected	
DNA Plasmid	GP + NP For 3 different strains (Zaire, Sudan, Ivory Coast)			4/4 protected	



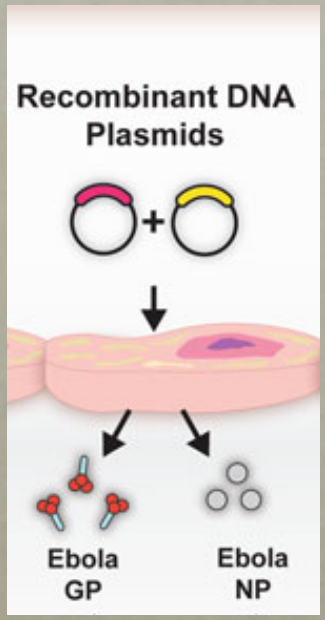
GP, glycoprotein; NP, nucleoprotein

Sullivan NJ, Sanchez A, Rollin PE, et al. Development of a preventive vaccine for Ebola virus infection in primates. *Nature*. 2000; 408: 605-609.

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Vaccines

Vaccine Vector	EBOV genetic material it carries	How vaccine given	Subjects	Study Results	Authors (Date)
DNA Plasmid	GP + NP	1 inject q4 weeks X3 doses Followed by ADV-ZEBOV GP booster 3 months later	NHPs	4/4 protected Produced higher Ab titers than ADV vaccine alone, but conferred no additional mortality benefit	Sullivan et al (2000)

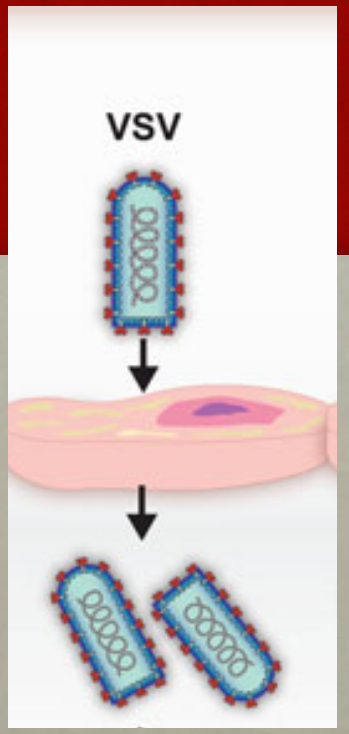


GP, glycoprotein; NP, nucleoprotein; NHP, non-human primates; ADV, adenovirus

Sullivan NJ, Sanchez A, Rollin PE, et al. Development of a preventive vaccine for Ebola virus infection in primates. *Nature*. 2000; 408: 605-609.

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Vaccine Vector	EBOV genetic material it carries	How vaccine given	Subjects	Study Results	Authors (Date)
Vesicular Stomatitis Virus	GP (VSV GP replaced with EBOV GP)	IM IN PO X1 dose 28 days before exposure	NHPs	100% protected	Geisbert et al (2011)
		X1 dose 30 min AFTER exposure		50-100% protected	
		X1 dose 1-2 days AFTER exposure			
		48h AFTER exposure	1 human	Survived accidental ZEBOV needlestick exposure	Choi et al (2013)
		Directly into CNS	NHPs	Vector did NOT produce neurotoxicity	Mire et al (2012)



GP, glycoprotein; NP, nucleoprotein; NHP, non-human primates; VSV, vesicular stomatitis virus

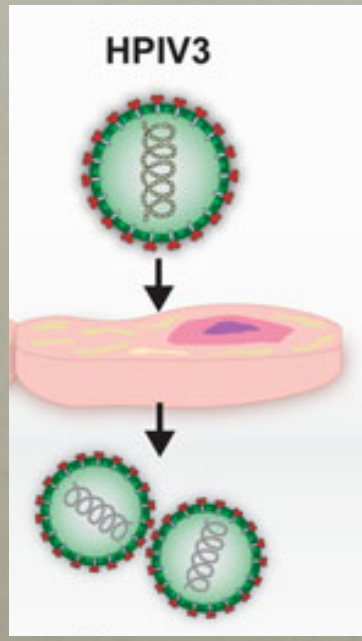
Geisbert TW, Feldman H. Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. The Journal of Infectious Disease. 2011; 204: S1075-S1081.

Mire CE, Miller AD, Carville A, et al. Recombinant vesicular stomatitis virus vaccine vectors expressing filovirus glycoproteins lack neurovirulence in nonhuman primates. *PLoS Negl Trop Dis*. 2012; 6(3): e1567.

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Vaccines

Vaccine Vector	EBOV genetic material it carries	How vaccine given	Subjects	Study Results	Authors (Date)
Human Parainfluenza Virus 3	GP GP + NP	1 dose q4wks x2doses	Guinea Pigs NHPs	100% protection	Choi et al (2013)



GP, glycoprotein; NP, nucleoprotein; NHP, non-human primates; HPIV3, human parainfluenza virus 3

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Vaccines

- Most vaccines studied are ZEBOV species-specific
- Multi-valent vaccines more valuable
  - Species arising during outbreaks difficult to predict
- Some multi-valent vaccines have been tested
  - 5/5 rhesus monkeys survived both ZEBOV and SUDV
  - 11/11 monkeys survived (each exposed to different strain)
- Difficulties
  - Limited cloning capacity of viral vectors

Geisbert TW, Geisbert JB, Leung A, et al. Single-injection vaccine protects nonhuman primates against infection with marburg virus and three species of Ebola virus. *J Virol*. 2009; 83(14): 7296-304.

Pratt WD, Wang D, Nichols DK, et al. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clin Vaccine Immunol*. 2010; 17(4): 572-81.

Passive Immunotherapy

Study Agent	Study Subjects	Result	Authors (Date)
Blood Transfusion from Survivors (=Convalescent-Phase Blood Donors)	Humans During 1995 Epidemic	7/8 survived Receiving better supportive care may have influenced	Mupapa et al (1999)
	NHPs	0/4 survived High Ab titers, but did not control viral replication	Jahrling et al (2007)
	Human (1) During 2014 Epidemic (U.S. Citizen)	Survived Receiving better supportive care may have influenced	---

Mupapa K, Massamba M, Kibadi K, et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International and Scientific Technical Committee. *J Infect Dis.* 1999; 179 (Suppl 1): S18-23.

Jahrling PB, Geisbert JB, Sweargengen JR, et al. Ebola hemorrhagic fever: evaluation of passive immunotherapy in nonhuman primates. J infect Dis. 2007; 196 (Suppl 2): S400-3.

Passive Immunotherapy

Study Agent	Study Subjects	How given	Result	Authors (Date)
Monoclonal Antibodies	Guinea Pigs	After exposure: • Before viremia developed • After viremia developed	10/10 survived when given before viremia 0/5 survived when given after viremia	Jahrling et al (1999)
	NHPs	24h after exposure + Day 4	0/15 survived Abs did not control explosive viral replication	Oswald et al (2007)

Jahrling PB, Geisbert TW, Geisbert JB, et al. Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infection. *J Infect. Dis*. 1999; 179 (Suppl 1): S224-34.

Oswald WB, Geisbert TW, Davis KJ, et al. Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. PLoS Pathog. 2007; 3(1): e9.

Passive Immunotherapy

Study Agent	Study Subjects	How given	Result	Authors (Date)
Monoclonal Antibodies ***(Triple Cocktail)***	Guinea Pigs	48h after exposure	6/6 survived	Qui, Fernando (2012)
		24h prior to exposure	3/6 survived	
	NHPs	1 dose q3days X3 doses Regimen started 24h after exposure	4/4 survived	Qui, Audet (2012)
		1 dose q3 days X3 doses Regimen started 48h after exposure	2/4 survived	
ZMapp®	Humans (2014 Outbreak)	Several days after symptom onset (9 days)	2/2 survived	---

Qui X, Fernando L, Melito PL, et al. Ebola GP-specific monoclonal antibodies protect mice and guinea pigs from lethal Ebola virus infection. PLoS Negl Trop Dis. 2012; 6(3): e1575.
Qui X, Audet J, Wong G, et al. Successful treatment of Ebola-virus infected cynomolgus macaques with monoclonal antibodies. *Science Translational Medicine*. 2012; 4(138): 138ra81.

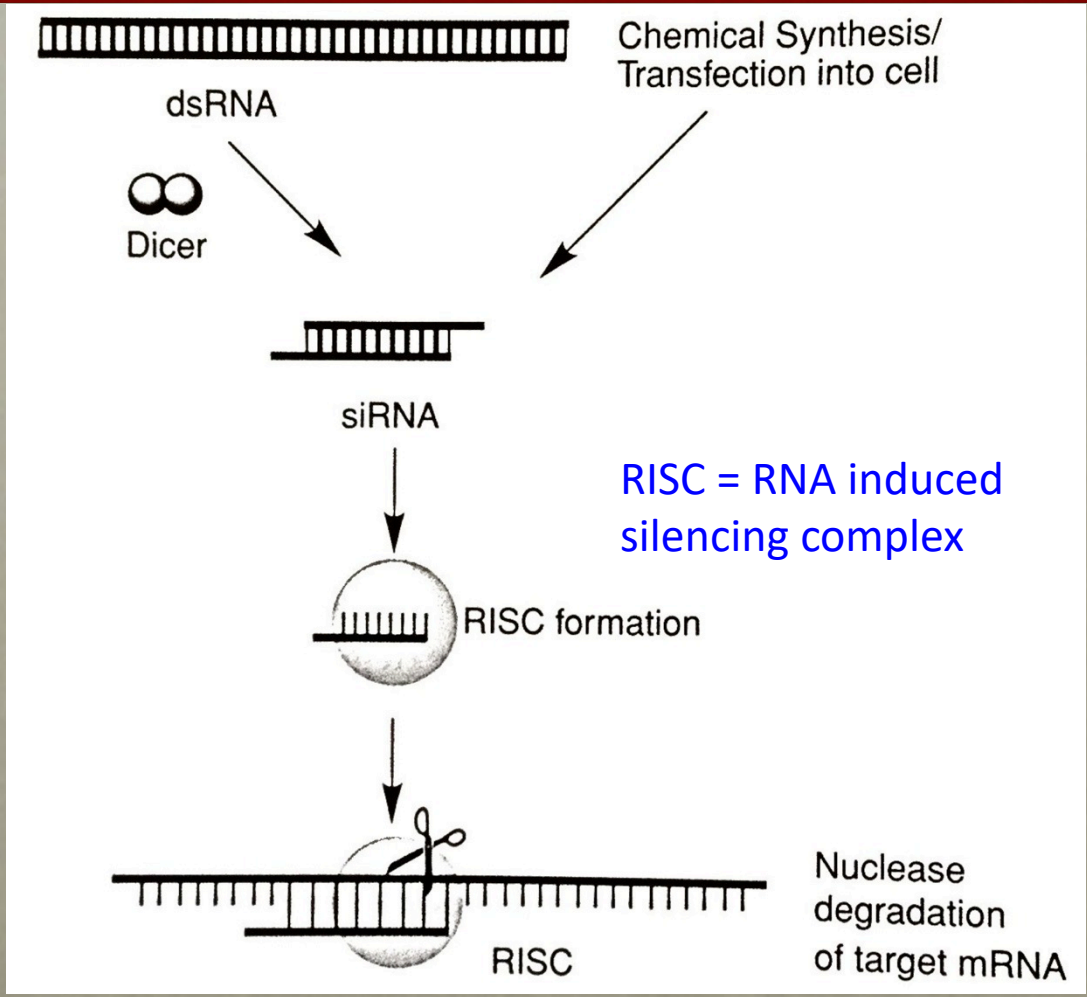
Passive Immunotherapy

- **Monoclonal Antibodies** (***Triple Cocktail***)
  - Molecular studies
    - MABs interact with Ebola GP at many different sites
  - Combinations of MABs are most effective
    - "Triple Cocktail" → 3 different MABs → each binds to distinct region of ZEOBV GP
    - Zmapp® → Ab's made in mice, then harvested
    - Neutralizes virus long enough for person's own immune system to take over

Qui X, Fernando L, Melito PL, et al. Ebola GP-specific monoclonal antibodies protect mice and guinea pigs from lethal Ebola virus infection. PLoS Negl Trop Dis. 2012; 6(3): e1575.
Qui X, Audet J, Wong G, et al. Successful treatment of Ebola-virus infected cynomolgus macaques with monoclonal antibodies. *Science Translational Medicine*. 2012; 4(138): 138ra81.

Viral RNA Interference

- Small-interfering RNA sequences
  - RNAi or siRNA
  - Introduction of small sections of RNA
    - Gets processed by host cell
    - Becomes associated with RISC
    - RISC + siRNA cleave viral mRNA targets
- siRNAs bound to lipid particles for stability
  - SNALPs = stable nucleic acid-lipid particles
  - LNP/siRNA = lipid nanoparticles
  - TKM-Ebola



Lemke TL, Williams DA, et al. (2008). *Foye's Principles of Medicinal Chemistry, 6th Edition* (p. 202). Baltimore, MD: Lippincott Williams.

Viral RNA Interference

Agent	Study Subjects	How Given	Results	Author (Date)
siRNA (via SNALP delivery)	Guinea Pigs	1h after exposure Then QD on days 1-6	5/5 survived 2 later died from drug toxicity	Geisbert et al (2006)
	NHPs	30 min after exposure Then QD on days 1-6	4/4 survived	Geisbert et al (2010)
(pooled siRNAs → 3 different ones w/ diff targets)	NHPs	30 min after exposure Then QD on days 1, 3, 5	2/3 survived	

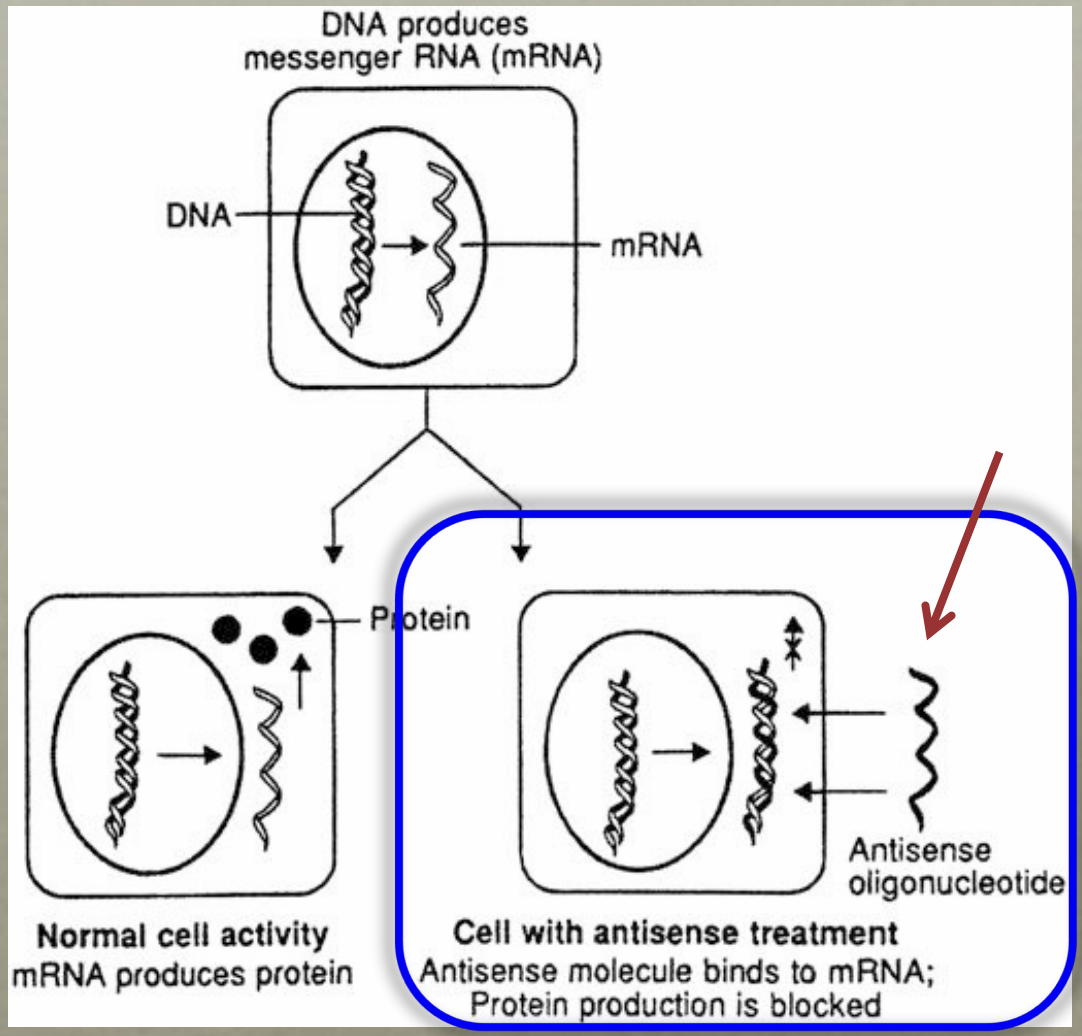
SNALPs = stable nucleic acid-lipid particles

Geisbert TW, Lee ACH, Robbins M, et al. *The Lancet*. 2010; 375: 1896-1905.

Geisbert TW, Hensley LE, Kagan E, et al. Post-exposure protection of guinea pigs against a lethal ebola virus challenge is conferred by RNA interference. *The Journal of Infectious Diseases*. 2006; 193: 1650-7.

Viral RNA Interference

- Complimentary RNA base sequences that bind to sections of EBOV RNA
  - Anti-sense phosphoro-diamidate morpholino oligomers
  - =PMO's
  - Steric hindrance
  - Prevents viral mRNA from making protein
  - Unique structure makes them soluble and stable in biological fluids
  - Multiple doses required



Lemke TL, Williams DA, et al. (2008). *Foye's Principles of Medicinal Chemistry, 6th Edition* (p. 202). Baltimore, MD: Lippincott Williams.

Viral RNA Interference

- PMO*Plus*
  - *"Plus"* → contains (+) charges throughout backbone to improve binding to viral RNA
  - AVI-6002
    - AVI-7537 + AVI-7539 (1:1 ratio)
    - Base sequences complimentary to regions coding for 2 proteins important for EBOV's virulence
      - VP24, VP35

Heald AE, Iversen PL, Saoud JB, et al. *Antimicrob. Agents Chemother., AAC Accepts*. Published online ahead of print. 25 August 2014.

Iversen PL, Warren TK, Wells JB, et al. Discovery and early development of AVI-7537 and AVI-7288 for the treatment of ebola virus and marburg virus infections. *Viruses*. 2012; 4: 2806-2830.

Viral RNA Interference

Agent	Study Subjects	How Given	Results	Author (Date)
AVI-6002 (PMO*Plus*)	NHPs	30-60 min after exposure Then QD x14 days	3/5 survived Suppressed viral load 100 fold	Warren et al (2010)
	Humans	Dose-escalation safety trail (Phase I Clinical Trial) Doses infused over 30 mins	30/30 Safe, well tolerated	Heald et al (2014)
PMO	NHPs	48h BEFORE exposure Then daily x9days	2/4 survived	Warfield et al (2006)

Heald AE, Iversen PL, Saoud JB, et al. Safety and pharmacokinetic profiles of phosphorodiamidate morpholino oligomers with activity against Ebola virus and Marburg virus: results of two single ascending dose studies. *Antimicrob. Agents Chemother., AAC Accepts*. Published online ahead of print. 25 August 2014.

Warren TK, Warfield KL, Wells J, et al. Advanced antisense therapies for postexposure protection against lethal filovirus infections. *Nature Medicine*. 2010; 16(9): 991-994.

Warfield KL, Swenson DL, Olinger GG, et al. Gene-specific countermeasures against Ebola virus based on anti-sense phosphorodiamidate morpholino oligomers. *PLoS Pathogens*. 2006; 2: 005-0013.

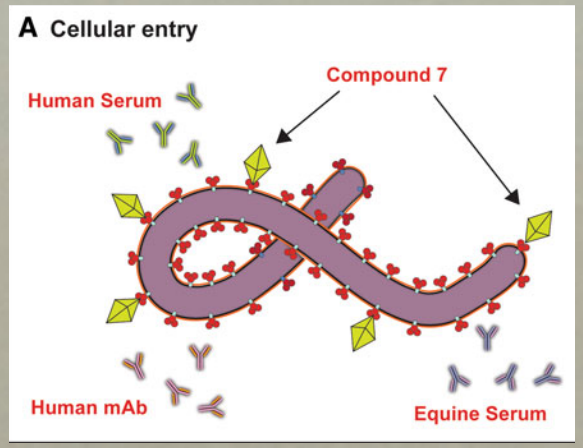
Viral RNA Interference

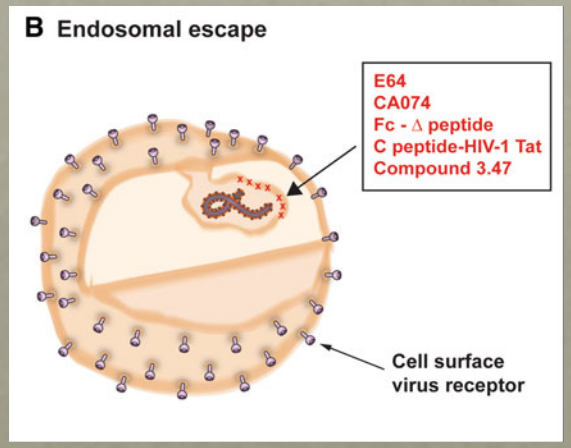
- BCX4430
  - New broad-spectrum anti-viral
    - Inhibits viral RNA polymerase
    - “RNA chain terminator”
  - High number of doses required for protection
    - 2 doses / day
    - x14 days
  - Drug-related toxicities
  - Protected monkeys exposed to Marburg filovirus infection
  - Not yet tested with EBOV

Wong G, Xiangguo Q, Olinger GG, et al. Post-exposure therapy of filovirus infections. Trends in Microbiology. 2014; 22(6): 456-463.

Inhibition of Other Viral Processes

- Most studies have proven efficacy *in vitro* only
- Limited *in vivo* studies available at this time
- BDZ-derivative ("Compound 7") fits in between hydrophobic pocket between GP1 and GP2 subunits on EBOV
  - Interferes with viral entry
- "Compound 3.47" binds to a protein responsible for EBOV viral entry
- A special peptide sequence conjugated to an IgG antibodiy shown to prevent EBOV viral release from endosome
- Cysteine Protease Inhibitors (E64 and CA074) shown to block processing of EBOV GP
  - Has as in vivo studies (mice)
  - Given pre-exposure: 80% mice protected
  - Given post-exposure: 50% mice protected





Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Protein C

- Dysregulation of clotting during EBOV infection
  - Overproduction of pro-coagulant tissue factors
    - Excess clotting → eventual depletion of clotting factors → bleeding
    - Disseminated Intravascular Coagulopathy (DIC)
- Protein C is an anti-coagulant
- Recombinant Nemotade Protein C2
- Recombinant Human Activated Protein C
- Phase II trials have conflicting results about safety and efficacy
- Only treats a symptom; not a cure for Ebola virus disease

Choi JH, Croyle MA. Emerging targets and novel approaches to ebola virus prophylaxis and treatment. *BioDrugs*. 2013; 27: 565-583.

Interferon-Beta Therapy

- EBOV disease causes increased IFN-alpha production, but not IFN-beta
  - Inefficient immune response against EBOV
  - Lymphocyte apoptosis
  - INF-alpha may have weaker immune cell receptor binding than INF-beta
- Post-exposure treatment w/ IFN-beta Increases survival time in monkeys
  - No mortality benefit
  - Possible adjunctive post-exposure therapy

Smith LM, Hensley LE, Geisbert TW, et al. Interferon-beta therapy prolongs survival in Rhesus models of Ebola and Marburg hemorrhagic fever. *The Journal of Infectious Diseases*. 2013; 208: 310-318.

Treatments

- NIH activities
  - Vaccines
    - Phase I Clinical Trials began in September 2014
  - Treatments
    - Contracted with Mapp Biopharmaceutical Inc (makers of ZMapp®)
      - Will begin Phase I Clinical Trials in near future
    - Working with one other company
- U.S. Department of Defense activities
  - Working with 1 company to develop a vaccine
  - Funded 2 companies for treatments

Centers for Disease Control and Prevention. 2014 Ebola outbreak in West Africa. http://www.cdc.gov/vhf/ebola/outbreaks/guinea. Accessed September 17, 2014.

ClinicalTrials.Gov

Study Agent registered at ClinicalTrials.gov	Description	Clinical Phase	Status
VRC-EBO-DNA-023-00-VP	DNA Plasmid Vaccine	Phase I (Healthy Humans, Uganda)	Completed (Feb 2010-Apr 2012)
VRC-EBO-DNA-023-00-VP	DNA Plasmid Vaccine	Phase I (Healthy Humans)	Completed (Jan 2008-June2010)
VRC-EBO-DNA-012-00-VP	DNA Plasmid Vaccine	Phase I (Healthy Humans)	Completed (Oct2003-Aug2007)

ClinicalTrials.Gov

Study Agent registered at ClinicalTrials.gov	Description	Clinical Phase	Status
VRC-EBO-ADV-018-00-VP	Multi-Valent Vaccine: • 2 Recombinant Adenovirus vectors (serotype 5) • 1 encodes for ZEOBV GP • 1 encodes for SUDV GP	Phase I (Healthy Humans)	Completed (Sept 2006-May 2009)
VRC-EBO-ADC-069-00-VP	Multi-Valent Vaccine: • 2 Recombinant Chimpanzee Adenovirus vectors (serotype 3) • 1 encodes for ZEOBV GP • 1 encodes for SUDV GP	Phase I (Healthy Humans)	Recruiting (Aug2014-Aug2016)

ClinicalTrials.gov

ClinicalTrials.Gov

Study Agent registered at ClinicalTrials.gov	Description	Clinical Phase	Status
AVI-6002	Post-exposure Prophylaxis Treatment • Anti-sense RNA oligonucleotide	Phase I (Healthy Humans)	Completed (May2010-Nov2011)
TKM-100802	Post-exposure Prophylaxis Treatment • Lipid Nanoparticle (LNP) • +Small-interfering RNA (siRNA)	Phase I (Healthy Humans)	Suspended (Jan2014-?) (Clinical hold placed on drug)

ClinicalTrials.gov

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- All appropriate isolation precautions are followed and ZB is started on IV fluids, ondansetron, and APAP in the ED.
- The ED attending physician comes to you again asking if there is any new treatment available on the market they can order and give to ZB. What is your response?

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- The ED attending physician says that he has heard about ZMapp on the news and asks you how it works. What is your response?

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- The ED attending physician is impressed with your knowledge of the disease, so he asks you if any treatments have been tested in humans yet. What is your response?

Patient Case

- ZB is a 30 yo white male who presents to the ED with a 5-day history of fever (T=39^{o}C), vomiting, watery diarrhea, bloodshot eyes, and muscle aches. Approximately 10 days ago, he returned from a medical missions trip to Liberia.
- ZB is treated for several days at [Hospital X] and eventually recovers. It is confirmed by laboratory PCR testing that ZB had ZEBOV infection. Could he become infected with Ebola virus again should he return to Africa?

Questions