Vaccines	Immunization Schedu <u>le</u>	Mouse Model	Guinea Pig Model	NHP Model
Virus Vectors				
 HPIV3 Immunogens HPIV3 ΔHN-F/ EBOV GP [1] EBOV GP [1-3] EBOV NP [2] EBOV GP + NP [3] EBOV GP +GM-CSF [3] 	 <u>Guinea Pigs</u>: IN 4 x 10⁶ PFU of HPIV3 ΔHN-F/EBOV GP or HPIV3/EBOV GP [1] IN 10^{5.3} PFU of HPIV/EBOV GP or NP [2] <u>HPIV3- NHPs</u>: IN plus IT 4 x 10⁶ TCID₅₀ of HPIV3/EBOV GP, HPIV3/EBOV GP, HPIV3/EBOV GP+GM-CSF, HPIV3/EBOVGP NP or 2 x 10⁷ TCID₅₀ of HPIV3/EBOV GP for 1–2 doses [3] 		 Complete protection with HPIV3 ΔHN-F/EBOV GP, HPIV3/EBOV GP, or HPIV3/EBOV NP [1, 2] Strong humoral response 	 Complete protection with 2 doses of HPIV3/EBOV GP [3] No advantage to bivalent vaccines
 RABV \(\Delta\GP\)/EBOV GP (Live attenuated) [4] RABV/EBOV GP fused to GCD of RABV (inactivated) [4] 	<u>Mice: I</u> M 5 x 10 ⁵ FFU	 Complete protection with either vector EBOV GP incorporation into virions not dependent on RABV GCD 		
Human Ad5 Immunogens • CMVEBOV GP [5-9] • CAGoptEBOV GP [8, 9]	Mice: • IN, PO, IM 1 x 10^{10} [6] to 5 x 10^{10} [5] particles of Ad5/CMVEBOV GP • IP 1 x 10^8 PFU Ad5/CMVEBOV GP[7] • IM 1 x 10^4 -1 x 10^7 IFU of Ad5/CMVEBOV GP or 1 x 10^4 -1 x 10^6 IFU of Ad5/CAGopt EBOV GP [9] <u>Guinea Pigs</u> : • IN, IM 1 x 10^{10} particles of	 With induced preexisting Ad5 immunity, complete protection with only IN Ad5/CMVEBOV GP [5] With no Ad5 immunity: complete protection regardless of route [5-7, 9] Mucosal immunization Ad5- EBOV GP increased cellular and humoral immunity compared to IM immunization[6] 	 With systemically induced preexisting Ad5 immunity, complete protection with IN Ad5/CMVEBOV GP [8] With mucosally induced preexisting Ad5 immunity, 83% protection with IN Ad5/CMVEBOV GP With systemically induced preexisting Ad5 	

Supplemental Table 1. Efficacy of vaccines in animal models of Ebolavirus disease.

Vaccines	Immunization Schedule	Mouse Model	Guinea Pig Model	NHP Model
	Ad5/CMVEBOV GP or Ad5/CAGopt EBOV GP[8]	 Complete protection with Ad5/CAGopt EBOV GP and 2 higher doses of Ad5/CMVEBOV GP [9] Increased cellular and humoral responses with Ad5/CAGoptEBOV GP 	 immunity, 78% or 100% protection with IM or IN CAGopt EBOV GP, respectively With no Ad5 induced immunity, complete protection regardless of route 	
AdC7/CMVEBOV GP[10]	<u>Mice</u> : IM 5 x 10 ⁹ –5 x 10 ¹⁰	 Complete protection; not 	Complete protection	
	particles of AdC7/EBOV GP	affected by induced	• Effect of preexisting Ad5	
	$\frac{Guinea Pigs}{10^{11}}$ particles/kg	preexisting AdS immunity	immunity not studied	
AdC5/1-CMVEBOV GP [11]	Mice: IM 5 x 10 ¹⁰ particles	Complete protection		
VSV ΔGP Immunogens	Immunocompetent Mice:	Complete protection with	 Complete protection 	 67% protection with
 EBOV GP attenuated 	• IP, IM, IN, PO 1–2 x 10 ⁴	VSV∆G/EBOV GP live vector	with homologous VSV	VSV ∆GP/EBOV GP in
[12-18] or irradiated[13]	PFU of VSV∆G/EBOV GP,	in immunocompetent mice	Δ GP/EBOV GP only [12]	HIV+ NHPs mediated
• TAFV GP [12]	TAFV GP, RESTV GP, or	[12, 13, 15]regardless of	83% cross EBOV species	by CD4+ cells [14]
RESTV GP	SUDV GP [12, 13, 15]	• Complete protection with	of VSV AGP/SUDV	Complete protection with homologous
SUDV GP SUDV GP+VP40	VSVΔG/EBOV GP [15]	VSVAG/EBOV GP given 7	GP+VP40	PO.[16] IN. [16]OR IM
• SUDV GP + NP	<u>NOD-SCID Mice</u> : IP 2 x 10^5	days prior to challenge	 IgG antibodies against 	[16, 17] VSV
SUDV GP+NP and SUDV	PFU of VSV∆G/EBOV GP	• No protection with irradiated	both SUDV antigens not	∆GP/EBOV GP to IM
GP+VP40	<u>Guinea Pigs</u> : IP 2 x 10 ⁵ PFU	vaccine [13]	increased after EBOV	[16, 18] or aerosol [17]
	of VSV∆G/EBOV GP, TAFV	• Complete cross-EBOV species	challenge indicating lack	EBOV challenge
	GP, RESTV GP, SUDV GP,	protection with	of viral replication	• 25% protection with
	GP+NP. SUDV GP+NP and		 NO EBOV neutralization antibodies detected 	following rechallenge
	SUDV GP+VP40 for 1–2	• 75% cross-EBOV species	following SUDV	with SUDV [18]
	doses [12]	protection with VSVΔG/SUDV	vaccination and EBOV	
	<u>HIV+ NHPs</u> : IM 1 x 10^7 PFU of	GP [12]	challenge	
	VSVAG/EBOV GP [14]	Complete protection in NOD-	Reduced cross-EBOV	
	• IM 1 x 10 ⁷ PELL of	SCID mice with high-dose	species protection with	
		V2V7Gb/FROM Gb [12]	SODV INP INCLUSION	

Vaccines	Immunization Schedule	Mouse Model	Guinea Pig Model	NHP Model
	 VSVΔGP/EBOV GP then EBOV challenge and SUDV rechallenge [18] PO,[16] IN,[16] IM[16, 17] 2 x 10⁷ PFU of VSVΔGP/EBOV GP 			
CMV Δm157/EBOV NP _{CTL} [19]	<u>Mice:</u> IP 5 x 10 ⁵ PFU–2 doses	 Complete protection 		
VV Immunogens • EBOV NP[20] • EBOV VP35 • EBOV VP40 • EBOV GP [20, 21] • EBOV sGP [20]	<u>Guinea Pigs</u> : SC 10 ⁷ of VV/EBOV NP, VP35, VP40, GP, OR sGP–3 doses[20] <u>NHPs</u> : SC of VV/EBOV GP–3 doses[21]		 60% protection with VV/EBOV GP only [20] Survival correlated with development of neutralizing antibodies 	 No protection with VV/EBOV GP [21] Viremia present in all subjects Time to death similar to controls
Virus-like Particles (VLPs)				
VEEV RNA replicon particles (VRP encoding: • EBOV NP [21-24] • EBOV GP [21, 23-26] • EBOV GP +NP[21, 23] • EBOV VP24 [24, 27] • EBOV VP30 • EBOV VP35 • EBOV VP40 • EBOV GP + Lassa GP combination [26] • EBOV GP/Lassa GP bivalent	Mice: • SC 2 x 10^6 FFU of VRP/EBOV NP3 doses [22] • SC 2 x 10^6 FFU or 2 x 10^6 IU of VRP/EBOV NP, VP24, VP30, VP35, or VP40 for 2-3 doses [24, 27] • SC 1x10 ⁶ IU of VRP/EBOV GP, NP, or GP+NP for 2 doses [23] • SC 1 x 10^8 of VRP EBOV GP- 4 doses [25] <u>Guinea Pigs</u> : • SC 10^7 IU of VRP EBOV GP, NP, or GP+NP-2 or 3 doses [23] • SC 10^7 IU of VRP EBOV	 75-100% protection with VRP/EBOV NP [22-24] 90–100% protection with VRP/EBOV GP [23-25] Complete protection with VRP/EBOV GP+NP [23] 95–100% with VRP/EBOV VP proteins in BALB/c mice[24] 100% protection with VRP/EBOV VP 30 or VP 35 proteins in C57BL/6 mice [24] 80% protection with VRP/EBOV VP40 in C57BL/6 mice [24] No protection with VRP/EBOV VP24 proteins in C57BL/6 mice [24, 27] 	 Strain 2 guinea pigs (2 doses): no protection with VRP-EBOV NP; 60% protection with VRP-EBOV GP [23] Strain 13 guinea pigs (3 doses): complete protection with VRP-EBOV NP+GP or VRP-EBOV GP; 20% protection with VRP- EBOV NP 80% protection with VRP- EBOV NP 80% protection with bivalent VRP/EBOV GP with Lassa GP[26] 100% protection with VRP/EBOV GP and VRP/Lassa GP 	 No protection with VRP/EBOV GP or NP or both immunogens [21] Viremia present in all subjects Time to death similar to controls

Vaccines	Immunization Schedule GP+Lassa GP-3 doses [26] <u>NHPs</u> : SC 2 x 10 ⁶ FFU of VRP EBOV GP, NP or GP+NP-3 doses [21]	Mouse Model	Guinea Pig Model	NHP Model
 KUN replicon encoding: EBOV GP [28] EBOV GP/Ctr EBOV GP mutant for ease of shedding (D637L) 	<u>Guinea Pigs</u> : IP 1 x 10 ⁶ or 5 x 10 ⁶ VLPs-2 doses		 >75% protection with KUN/GP or KUN/GP mutant at higher dosage 50% protection with KUN/GP mutant at lower dosage 25% protection with KUN/GP at lower dosage No protection with KUN/soluble GP 	
rBV encoding: • EBOV VP40 • EBOV GP [29] rBV replicon encoding: • EBOV VP40 • EBOV GP • EBOV NP [30] 293T cell-derived EBOV VP40 + GP + NP [30]	 Mice: IM 1, 10 or 50 ug of rBV or 293T cell derived/EBOV VP40 + GP + NP VLPs-2 doses [30] IM 10 or 50 μg of rBV/EBOV VP40 + GP VLPs-2 doses [29] IM 10 ug of rBV/EBOV VP40 GP VLPs-3 doses 	 Dose dependent protection with 2-dose regimen; complete protection at highest dose [29, 30] 83% protection with 3 doses[29] Equivalent immune responses and protection from challenge with rBV- derived or 293T cell-derived VLPs[30] 		
Liposomes encapsulating: • EBOV GP + VP40 [31-33] • EBOV NP+GP+VP40 [34, 35] • EBOV GP + MARV VP40 [31] • EBOV VP40 +MARV GP	<u>Mice</u> : IM, IP 0.1, 1, or 10 μg EBOV GP + VP40 VLPs for 2 [33] or 3 doses [32] <u>STAT-1 KO mice</u> : IM 10 μg EBOV GP + VP40 + NP–3 doses [34] <u>Guinea Pigs</u> :	 Complete or nearly complete protection with highest dosage EBOV GP + VP40 VLPs in BALB/c,[32] C57BI/6,or perforin- deficient mice [33] 50% protection in CD4+- 	 Complete protection with EBOV GP + VP40 VLPs [31] 90% protection with EBOV GP + MARV VP40 VLPs or equal mixture of EBOV GP + VP40 and MARV GP + VP40 VLPs 	 Complete protection with EBOV NP+GP+VP40 [35] Viremia and clinical or laboratory signs of EBOV infection not detected in vaccinated

Vaccines	Immunization Schedule	Mouse Model	Guinea Pig Model	NHP Model
• MARV GP + VP40	 IM 100 μg of EBOV GP + VP40 VLPs [31] IM 50 μg EBOV GP + VP40 and 50 μg MARV GP + VP40 VLPs <u>NHPs</u>: IM 250 μg EBOV NP + GP + VP40 VLPs in RIBI adjuvant-3 doses [35] 	 deficient mice 13% protection in IFN-γ- deficient mice No protection in B cell-, βδ TCR-, CD8+-, or STAT-1- deficient mice[33, 34] 	 No protection with EBOV VP40 +MARV GP High antibody titers and no viremia in survivors 	 and challenged NHPs Strong antibody and T cells (tumor necrosis factor-α) responses
DNA Vaccines				
 DNA Plasmid Immunogens EBOV GP [36-40] EBOV GP glycosylation deletions[37] EBOV sGP[39] EBOV NP [36, 39, 40] EBOV GP + NP [38] EBOV GP + TAFV GP+ SUDV GP + EBOV NP EBOV GP, Marv GP, VEEV 26s, Anthrax PA [40] 	 <u>Mice</u>: Prime-0.5 μg of EBOV GP DNA, then 3 boosts with 1.5 μg via gene gun [36] 0.5 or 3 μg of EBOV NP or GP DNA via gene gun–3 doses [36] 0.25–0.5 ug EBOV GP and glycosylation mutants DNA via gene gun–3 doses [37] 5 ug EBOV GP or NP DNA via gene gun-2 doses [40] <u>Guinea Pigs</u>: 5 μg of EBOV GP DNA via gene gun-3 doses [40] 5 μg each of EBOV GP, MARV GP, VEEV 26s, Anthrax PA DNA via gene gun–3doses[40] IM 100 μg of EBOV GP DNA + 25 μg of EBOV GP DNA + 25 μg of EBOV NP DNA–3 doses [38] 	 Complete protection with EBOV GP DNA prime/boost [36] Similar dose-dependent partial protection (~60–90%) with either EBOV NP or GP DNA with boosts[36] 89% protection with EBOV GP DNA [37] 29–31% protection with deletion of mucin region or an N-linked GP2 glycosylation site involved in dimerization of GP1 and GP2 IgG antibody titer generally correlated with protection with EBOV GP or glycosylation mutants DNA Complete protection with 2 doses of EBOV GP or NP DNA; high antibody response[40] 	 Complete protection with EBOV GP DNA alone[38, 39] or in combination with NP, or EBOV NP in combination with TAFV GP, EBOV GP SUDV GP and EBOV GP DNA[38] Complete protection with EBOV NP or GP DNA and 83% protection with EBOV sGP DNA if challenge was within 2 months following first immunization [39] Lower protection and antibody titers to immunogens if time to challenge nearly doubles 67% protection with EBOV GP DNA[40] 60% protection with multivalent EBOV GP, Marv GP, VEEV 26s, Anthrax PA DNA 	

Vaccines	 Immunization Schedule IM 25 μg each of DNA plasmids encoding EBOV GP, TAFV GP, SUDV GP, and EBOV NP–3 doses IM 50 μg of EBOV GP, sGP, or NP DNA–4 doses [39] 	Mouse Model	Guinea Pig Model	NHP Model
DNA Prime, rBV boost[41] • EBOV GP • EBOV GP/Ctr	 <u>Guinea Pigs</u>: Prime: 2.5 μg EBOV GP DNA via gene gun, then 2 boosts: SC 5 μg of rBV- derived EBOV GP or GP/Ctr Prime and 2 boosts of rBV EBOV GP or GP/Ctr or EBOV GP DNA 		 50% protection with prime and boosts of rBV EBOV GP 33% protection with DNA prime, rBV EBOV GP/Ctr boosts 17% protection with prime and boosts of EBOV GP DNA or rBV EBOV GP/Ctr No protection with prime with EBOV GP DNA and boosts with rBV EBOV GP 	
Fusion Proteins				
RESTV GP1 fused to GP1 mAb forming immune complex [25]	 Mice: SC 10 μg of immune complex alone or with PIC and/or alum adjuvant-4 doses SC 10 or 25 μg of immune complex with adjuvant-3 doses 	 80% protection with 4 doses of immune complex and PIC with or without alum adjuvant 50% protection with 3 doses of immune complex (25 μg) plus both adjuvants 20% protection with3 doses of immune complex (10 μg) plus both adjuvants 		
RESTV GP fused to Fc lgG1 fragment [42]	<u>Mice</u> : IP 100 μg fusion protein in complete Freund's adjuvant, then	 88% protection with fusion protein 13% protection with Fc-FLAG 		

Vaccines	Immunization Schedule boost with 25 μg in incomplete Freund's adjuvant–3 doses	Mouse Model epitope tag	Guinea Pig Model	NHP Model
Ebolavirus Vaccines				
EBOV • live[43-46] • irradiated [21, 32, 47] • irradiated, in liposomes [21, 47] • INA+ UV irradiated, MA [48]	 Mice SC, IM, ID 100 PFU MA- EBOV prior to IP challenge [43-46] IP 10 μg of irradiated EBOV-3 doses [32] IM, IV 1.4 μg of irradiated EBOV alone or in liposome-2 doses [47] IM 5 x 10⁴ PFU of INA inactivated MA-EBOV-1 or 2 doses [48] NHPs: IV 194 μg of EBOV encapsulated in liposome- 3 doses [21, 47] SC 50 μg of irradiated EBOV-3 doses[21] 	 Complete protection with SC, IM live EBOV; [43-45] protection dependent on CD8+ cells and interferon- α/β receptor and not on B or CD4+ cells [45, 46] Persistent infection in CD4- depleted or B cell-deficient mice[46] 25, 45, or 55% protection with IP, IM, or IV irradiated EBOV [32, 47] Complete protection with IV irradiated liposome- encapsulated EBOV[47] 77% protection with IM liposome encapsulated EBOV >80% protection with INA- inactivated EBOV[48] 		 No protection with liposome encapsulated EBOV; viremia present [21, 47] 25% protection with rradiated EBOVin macaques; viremia present in all macaques[21] Neutralizing antibody titers present in 1 surviving macaque immunized with irradiated EBOV
EBOV ΔVP30 [49]	<u>Mice</u> : IP 10 ⁶ FFU–2 doses <u>Guinea Pigs</u> : IP 10 ⁷ FFU–2 doses	Complete protection correlated with cellular and humoral responses	Complete protection	species. CMV: cytomegalovirus

Abbreviations: AD: adenovirus, CAGopt: cytomegalovirus early enhancer element and chicken beta-actin promoter optimized, TAFV: Cote d'Ivoire ebolavirus species, CMV: cytomegalovirus promoter, CTL: Cytotoxic T lymphocytes, Ctr: C terminal truncation, EBOV: Zaire ebolavirus species, F: fusion protein, FFU: focus-forming units, FLAG: DTKDDDDK peptide fused to Fc region of IgG1, GCD: glycoprotein cytoplasmic domain, GM-CSF: granulocyte macrophage colony stimulating factor,GP: glycoprotein, GPA: guinea pig adapted, HIV: human immunodeficiency virus, HN: hemagglutinin-neuraminidase, HPIV3: human parainfluenza virus type 3, ID: intradermal, IFU: infectious units, Ig: Immunoglobulin, IFN-γ: interferon gamma, IM: intramuscular, IN: intranasal, INA: 1,5-iodonaphthylazide, IP: intraperitoneally, IT: intratracheal, IV: Intravenous, KO: knockout, KUN: Kunjin, MA: mouse adapted, mAb: monoclonal antibody, MARV: Marburg virus, NHP: nonhuman primate, NOD: nonobese diabetic, NP: nucleoprotein, PA: protective antigen, PFU: plaque-forming units, PIC: polyinosinic:polycytidylic acid, PO: oral, RABV: rabies virus, rBV: recombinant baclovirus, RESTV: Reston ebolavirus species, RNA: ribonucleic acid, SC: subcutaneous, SCID: severe combined immunodeficiency, SUDV: Sudan ebolavirus species, sGP: soluble glycoprotein, STAT-1: signal transducer and activator of transcription-1 protein, TCID: tissue culture infective dose, TCR: T cell receptor, Th1: T helper cells 1 subset, VEEV: Venezuelan equine encephalitis virus, VLP: virus-like particles, VP: viral protein, VRPs: VEEV RNA replicon particles, VSV: vesicular stomatitis virus, VV: vaccinia virus

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
EBOV • Live [50] • Irradiated [51] • INA- inactivated, MA [48]	 Mice SC 10¹-10⁶ PFU EBOV - 18-48 hours prior to IP exposure[50] IP 5 x 10⁴ PFU of INA - inactivated EBOV -3 days [48] IP 25 μg of irradiated EBOV -3 days 	 Protection dependent on SC dose and time to challenge; complete protection at highest dose and greatest time interval to challenge[50] Complete protection with inactivated EBOV [48] No protection with irradiated EBOV [51] 		
Virus Vectors				
Ad5 Immunogens [9] • CMVEBOV GP • CAGopt EBOV GP	<u>Mice</u> : IM 5 x 10 ⁷ IFU +30 minutes	 Complete protection with AD5/CAGopt EBOV GP 22% survived with AD/CMVEBOV GP 		
VSV ΔGP Immunogens • EBOV GP [52] • SUDV GP [53]	<u>Mice</u> : IP 2 x 10^5 VSV ΔGP/EBOV GP PFU -1 day or +30 minutes or 1 day [52] <u>Guinea pigs</u> : IP 2 x 10^5 VSV ΔGP/EBOV GP PFU -24 hours or +1 or 24 hours [52] <u>NHPs</u> : IM 2 x 10^7 PFU of VSV ΔGP/EBOV GP [52] or VSV ΔGP/SUDV GP +20– 30 minutes postexposure to EBOV or SUDV [53]	 Complete protection with VSV ΔGP/EBOV GP regardless of time of treatment [52] Mild weight loss on +1 day, suggesting viral replication 	 66, 83, or 50% protection with VSV ΔGP/EBOV GP 24 hours prior to or 1 or 24 hours after challenge, respectively [52] 	 50% protection with VSV ΔGP/EBOV GP +20–30 minutes [52] Complete protection with VSV ΔGP/SUDV GP +20– 30 minutes [53] Control subject receiving VSV ΔGP/LASV GP lived for 17 days after SUDV challenge
Virus-like Particles (VLPs)				

Supplemental Table 2. Efficacy of Peri-exposure Treatment in Animal Models of EVD.

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
 EBOV GP + VP40[51] EBOV VP40 	 Mice: IM, IP 25 μg of EBOV GP + VP40 VLPs -1-3 days 10 μg of EBOV VP40 VLPs -3 days 	 80–100% protection EBOV GP + VP 40 Complete protection with EBOV VP40 VLPs only; not dependent on presence of EBOV GP 15–17% protection in NK cell-deficient or -depleted mice 		
Passive Immunity				
Pooled immune serum to live EBOV [43, 54]	Immunocompetent mice: IP 1 mL of antisera (anti- EBOV IgG titers of ≥6,400) _1 day or + 1 day [43] <u>SCID mice</u> : IP 1 mL of antisera (anti-EBOV IgG of ≥400,000 titers) -1 day <u>NHPs</u> : IV 6 mL/kg whole blood immediately after challenge and +3 or 4 days (anti-EBOV IgG ELISA titers of 100,000) [54]	 89% protection in immunocompetent mice pretreated with immune serum [43] Complete protection in immunocompetent mice from postchallenge treatment with immune serum Complete protection in SCID mice pretreated with immune serum Protection correlated with anti-EBOV IgG titers 		 No protection or delay in death compared to controls [54] Rapid decline of anti- EBOV IgG titers by day +3 Comparable viremia in treated and control NHPs
Pooled immune serum to VSV ΔGP/EBOV GP [15]	<u>Mice</u> : IP 0.5 mL of immune serum -1 day	 80% protection with pooled immune serum; neutralizing antibody titers equivocal 		

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
Pooled Immune serum to VLPs expressing: • EBOV NP [22, 23] • EBOV GP [23, 27] • EBOV VP24 [27] • EBOV VP30 • EBOV VP40 • EBOV VP40 + GP[33]	 Mice IP 0.8 mL of antisera (~4 log₁₀ ELISA titer) to VRP/EBOV GP or NP-9 hours [23] IP 1mL of antisera to VRP/EBOV NP (2.5-3 log₁₀ ELISA titer), [22] VRP/EBOV GP, VRP/EBOV VP24, VRP/EBOV VP30, or VRP/EBOV VP40 (~4 log₁₀ ELISA titer) [27] -1 day [22, 27] IV 0.5 mL of antisera to VLP/EBOV VP40 and GP -3 days [33] Guinea pigs: IP 5 mL of antisera (4 log₁₀ ELISA titer) to VRP/EBOV GP -3 hours [23] 	 10% protection with antisera to VRP-EBOV NP [23] 75–85% protection with antisera to VLP-EBOV GP [27] not confirmed with antisera with same ELISA titers in similar study [23] No protection with antisera to VLP/EBOV VP40 and GP, [33] VRP/EBOV NP, [22], or VRP-EBOV VP proteins [27] Lack of protection could be due to a poor CTL response (not measured) 	 20% protection with transfer of immune serum to VRP/EBOVGP [23] Cell mediated protection may be more important than humoral responses 	
 Purified polyclonal IgG antibody against: live EBOV [43] DNA and rAd5 EBOV GP vectors [55] Unknown, EBOV- immunized horses [56, 57] 	<u>Mice</u> : • IP 1 mL of purified mouse IgG (>100,000– 400,000 anti-EBOV IgG titers) -1 day [43] • SC 0.03, 0.3, 3 mL/kg horse IgG +20–30 minutes [57] <u>Guinea Pigs</u> : IM 1 mL/kg	 40-66% protection with mouse IgG[43] Efficacy of protection with IgG antibodies is titer dependent Similar protection with polyclonal IgG or immune serum transfer with equivalent IgG titers 	 Complete protection with horse IgG given at day 0 only; no viremia detected [57] Complete protection with horse IgG with second dose at day +3; viremia not detected No protection if IgG is 	 25% protection with IgG from NHPs immunized with DNA and rAd5 EBOV GP vectors [55] No protection with horse IgG immediately postchallenge[56, 57] or - 2 days [57] Delayed viremia with

Peri-exposure Treatment	Dose and Route of	Mouse Model	Guinea Pig Model	HP Model
	Administration several minutes and +3 days postexposure or +4 days only[57] <u>NHPs</u> : • IV 160-600 mg NHP lgG/kg -6 or 16 hours [55] • IM ~1 mL/kg of horse lgG (log serum neutralization index of 4.2) immediately after challenge [56, 57], or -2 days prior to or day 0 and day +5 [57]	• 25% protection with horse IgG at highest dose only; Iower doses not effective [57]	delayed until day +4; transient reduction in viremia and anti-EBOV titers not detectable	reduction in anti-EBOV titers with NHPs receiving IgG immediately after challenge; no delay in death • 33% protection with 2 doses of horse IgG
 mAb EBOV GP-specific Mouse IgG2a [58, 59] Mouse IgG1 [59, 60] Mouse IgG2b [59]Human IgG1[61, 62] Chimeric human IgG1- mouse IgG2a [63] 	 Mice: IP 25, 50, 100 μg of mAB lgG2a or lgG1 -1 day or +1-2 days [58] IP 2-256 μg of mouse lgG1 -1 day and +2 days [60] IP 100 μg of mouse lgG1 ±1 day [59] IP 100 μg of mouse lgG1 -1 day or +2-4 days [60] IP 3 μg or greater of chimeric glycoforms -1 day [63] Guinea Pigs: IP 0.5, 5, 50 mg/kg of human lgG1 	 Dose-dependent protection with mouse IgG2a or IgG1 [58, 60] Mouse IgG2a more effective (20-100%) than IgG1 (0-60%) given ±1 day [58]; not confirmed in recent study [59] Protection not correlated with neutralizing capacity [58, 59] Mouse IgG2a, IgG2b, or IgG1 more effective given after challenge than before challenge [59] 75–88% protection with 1 dose of mouse IgG1 given prior to or +2 days [60] 	 No protection when Human mAb given +6 hours [61] 100% protection at highest dose (50 mg/kg) when human mAb given at time of challenge or - 1 hour (25 mg/kg) 80% protection if human mAb given +1 hour 25–66% protection with mouse lgG1 133/3.16 given -1 day or +2 days after challenge; little protection with mAb 266/8.1 [60] 	 No protection with human mAb [62] Minimal effect on EBOV viral replication Cellular immunity may be needed for protection

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
	 (neutralizing) several minutes postchallenge [61] IP 25 mg/kg of human lgG1 -1 hour or +1 or 6 hours IP 20–25 mg/kg of mouse lgG1 given -1 day or up to +2 days [60] IP 10–12.5 mg/kg of mouse lgG1 on days +1, 3, 5, 7, and 9 <u>NHPs</u>: IV 50 mg/kg -1 day and +4 days [62] 	 Dose-dependent protection with chimeric mouse human mAb fucose- free glycoform [63] 	• Multiple lower doses of mouse IgG1 postexposure not improve protection compared to higher dose close to challenge	
Murine immune components to VLPs/EBOV VP40 and GP: • Immune serum • Splenocytes • T or B cells [33]	<u>Mice</u> : IV 2 x 10 ⁷ unfractionated splenocytes and/or 0.5 mL of immune serum or1 x 10 ⁷ B or T cells -3 days	 No protection with splenocytes, immune serum, or T or B cells from immunized mice 90% protection with transfer of both immune serum and splenocytes 		
CD8+ T cells from mice vaccinated withVRP/EBOV GP, NP, or VP proteins and restimulated with peptides fromthese proteins [24] T cells (CD4+-or CD8+- enriched, or unfractionated T cells from VRP EBOV NP	<u>Mice</u> : IP 1 x 10 ⁴ –8 x 10 ⁶ T cells -4 hours [22, 24]	 Complete protection with unfractionated T cells [22] Protection with CD8+ cells dependent on epitopes present in EBOV proteins and MHC class I molecules expressed by different mouse strains[24] No protection with CD4+- enriched cells [22] 		

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
vaccinated mice[22]				
NK cells stimulated with EBOV GP + VP40 or EBOV VP40 VLPs [51]	<u>Mice</u> : IP adoptive transfer of 5 x 10 ⁶ stimulated NK cells -6 hours	 70% protection with transfer of NK cells stimulated with EBOV GP + VP40 VLPs; dependent on perforin, not dependent on IFN-γ 100% protection with transfer of NK cells stimulated with VLPs/EBOV VP40 		
Antiviral Agents				
FGI-103 [64]	 Mice: IP 10 mg/kg -1 hour and days +2 and 5 IP 5 or 10 mg/kg +1 day IP 10 mg/kg once +1-5 days 	 Complete protection with 3 doses Dose-dependent protection (60-100%) with single dose +1 day No protection with single dose +2 or more days 		
FGI-106 [65]	 Mice: IP 01, 0,5, 1, 2, 5 mg/kg of FGI-106 -1 hour and +24 and 72 hours postchallenge[65] IP 5 mg/kg given on day +1; days +1 and 5; or days 0, +1, 3, and 5 IP 0.5, 1, 5 mg/kg single dose on day +1 	 Dose-dependent protection when given before and after challenge; complete protection with 2 higher dosages Complete protection with 5 mg/kg given on day +1 and 5, and day 0, +1, 3, and 5 Dose dependent protection with single doses given +1 day; 90% protection with single 1 or 5 mg/kg dose 		

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
		 Dramatic reduction in tissue viral load 		
Cyanovirin-N [66]	<u>Mice</u> : SC 0.48, 1.4, 4.8, or5.6 mg/kg -1 day before or at challenge, then daily for +5-8 days	 20–40% protection; mean time to death increased at 2 higher dosages 		
3-DeazaneplanocinA [45, 67]	 Immunocompetent Mice: SC 1 mg/kg +1 hour or +1 or 2 days [45, 67] or +3 or 4 days [67] SC 1 mg/kg day +1, days +1-2, or days +1-3 [67] SC 0.125, 0.25, 0.5, or 1 mg/kg given once +1 day [67] SCID mice: SC 1 mg/kg single dose day 0-3 postchallenge or days +1-15 [67] 	 Dose-dependent protection (40-100%) in immunocompetent mice treated with single dose[67] Complete protection in immunocompetent mice if treated once within 2 days of challenge; [45, 67] dependent on IFN-α/β [45] Delay of death in SCID mice with single or multiple doses postinoculation [67] 		
Carbocyclic 3- deazaadenosine[67, 68]	 Immunocompetent Mice: SC 10, 20, 40, 80 mg/kg once on day +1 [67] SC 80 mg/kg single dose on day 0–4 [67] IP 0.03–20 mg/kg initiated -1 day continuing every 8 hours for 9 days [68] IP 2.2, 6.7, or 20 mg/kg initiated 0–3 days continuing every 8 hours for 5–9 days 	 Dose-dependent protection (0-100%) in immunocompetent mice given a single dose[67, 68] Nearly complete protection with single SC dose (80 mg/kg) given on days +1-2 [67] Reduction in viral titer greatest with single dose given on day +2 Delay of death in SCID mice Complete protection with 		

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
	<u>SCID mice</u> : SC 80 mg/kg single dose on day 0–3 [67]	IP dosages ≥0.7 mg/kg initiated -1 day [68] Complete protection with 2 lower IP dosages given at day 0 or +1 day		
rhuman mannose-binding lectin (rhMBL) [69]	<u>Mice</u> : IP 75 or 350 μg of rhMBL every 12 hours for 10 days initiated _1 hour or +12 hours	 No protection with lower dosage >40% protection with pre- or post-treatment No protection in C3 KO mice 		
Antisense Phosphorodiamidate morpholino oligomers (PMOs)[70-73]	 Mice: IP 5, [72] 50,[72] or 500 [70, 72] μg of PMO targeting VP35 at -24 and 4 hours IP 1, 5, or 50 [71, 72] or 500 ug[72] of PMOs targeting VP24 at -24 and 4 hours [71, 72] IP 5, 50, or 500 μg of 3 PMOs targeting VP24, VP35 or L -4 hours or +24 hours [72] IP 50 or 500 μg of PMO targeting L L -4 hours or - 24 hours IP 10 mg/kg of PMO with piperazine moieties targeting VP24 and VP35 -24 hours or +24, 48, 72 or 96 hours [73] 	 Complete protection following pretreatment with 500 μg (2 doses) of PMO targeting VP35 [70, 72] 100% protection following pretreatment with 2 higher doses PMOs targeting VP24, depending on location of homologous sequences [71] Nearly complete protection following pretreatment with 500 ug of PMO targeting VP24[72] Complete protection with highest dose of 3 PMOs each targeting VP24,VP35, or L either pre- or postexposure[72] ~30% protection with 	 <75% protection with combination of PMOs each targeting VP24, VP35, or L given +4 days; protection lower with given preexposure or +1 day [72] Reduction in viral titer correlated with survival 	 50% protection with PMOs targeting VP24+VP35+L [72] High anti-EBOV antibodies and T cell responses in survivors No protection with PMO targeting VP35 only 62.5% protection with SC and IP piperazine- enriched PMOs targeting VP24 and VP35 [73] Reduced viremia and release of IL-6 and MCP-1 with PMOs targeting VP24 and VP35 Dose dependent protection (0-60%) with IV PMOs targeting VP24 and VP35 100 times lower viral

Peri-exposure Treatment	Dose and Route of Administration	Mouse Model	Guinea Pig Model	HP Model
Antisense PMOs (continued)	 <u>Guinea Pigs</u>: IP 10 mg of each PMO targeting VP24, VP35 or L -1 day or +1 or 4 days [72] <u>NHPs</u>: SC, IP, and IM of PMO(s) targeting VP35 or VP24+VP35+L -2 days through +9 days [72] SC and IP of piperazine- enriched PMOs 40 mg/kg targeting VP24 and VP35 30–60 minutes after challenge then daily for +10 or 14 days[73] IV 4, 16, 28, or 40 mg/kg of PMOs targeting VP24 and VP35 30–60 minutes after challenge then daily for +14 days 	 following pretreatment with 500 ug of PMO targeting L Complete protection following pretreatment with PMO targeting VP24 and VP35 [72, 73] Postexposure protection diminishes with delay of administration of piperazine-enriched PMOs targeting VP24 and VP35[73] 		titers in treated NHPs than in NHPs receiving PMO targeted to MARV proteins
Small interfering RNA (siRNA) encapsulated in liposomes [74, 75]	 Guinea Pigs: IP 8 mg/kg of a pool of 4 siRNAs targeting L in polyethylenimine -3 hours prior to challenge then +1, 2, and 4 days [74] IP 0.75 or 1 mg/kg of pool of 4 siRNAs targeting L in SNALP +1 hour then daily for +6 days 		 20% protection with polyethylenimine- delivered siRNAs targeting L gene; reduction in viremia following siRNA administration [74] 60% protection with higher dosage of SNALP- delivered siRNAs targeting L gene Complete protection 	 66% protection with 4 doses of siRNAs targeting EBOV L, VP 24, and VP 35; induced mRNA cleavage at target sites; no viremia in survivors[75] Complete protection with 7 doses of siRNAs; low EBOV viremia in treated animals

Peri-exposure Treatment	Dose and Route of	Mouse Model	Guinea Pig Model	HP Model
	Administration			
siRNA encapsulated in	NHPs:	-	with lower dosage of	
liposomes (continued)	 IV 2 mg/kg of pool of 		SNALP-delivered siRNAs	
	siRNAs targeting EBOV L,		pool; no viremia	
	VP 24, and VP 35 in		detected	
	SNALP +30 minutes, day			
	+1, 3, and 5 after			
	challenge–4 doses total			
	[75]			
	 IV 2 mg/kg of pool of 			
	siRNAs in SNALP +30			
	minutes and daily for			
	days +1–6 –7 doses total			
Abbreviations: AD: adenovirus, CAGopt: cytomegalovirus early enhancer element and chicken beta-actin promoter optimized, CMV: cytomegalovirus promoter, CTL: Cytotoxic T lymphocytes, EBOV: Zaire ebolavirus species, ELISA: enzyme-linked immunosorbent assay, FGI-103: (2-(2-(5-amino(imino)methyl)-1-benzofuran-2-yl)vinyl)-1H-benzoimidazole-5-				

lymphocytes, EBOV: Zaire ebolavirus species, ELISA: enzyme-linked immunosorbent assay, FGI-103: (2-(2-(5-amino(imino)methyl)-1-benzofuran-2-yl)vinyl)-1H-benzoimidazole-5carboximidamide), FGI-106: (quino [8,7-h] quinoline-1,7diamine,N,NI2-bis [3-(dimethylamino)propyl]-3,9-dimethyl-, tetrahydrochloride), GP: glycoprotein, Ig: Immunoglobulin,IL-6: interleukin-6, IFN: interferon, IM: intramuscular, INA: 1,5-iodonaphthylazide, IP: intraperitoneally, IV: intravenous, KO: knockout, mAb: monoclonal antibody, L: L polymerase, LASV: Lassa virus, MCP-1: monocyte chemotactic protein-1, MHC: major histocompatibility complex, NHP: nonhuman primate, NK: natural killer cells, NP: nucleoprotein, PFU: plaque-forming units, PMO: antisense phosphorodiamidate morpholino oligomers, rhMBL: recombinant human mannose-binding lectin, RNA: ribonucleic acid, SC: subcutaneous, SCID: severe combined immunodeficiency, SUDV: Sudan ebolavirus species, siRNA: small interfering RNAs, SNALP: stable nucleic acid lipid particle, VLP: virus-like particles, VP: viral protein, VRPs: VEEV RNA replicon particles, VSV: vesicular stomatitis virus

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