

DNA VACCINES FOR BIODEFENSE

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ATTRIBUTES OF DNA VACCINES

Advantages Over Conventional Methods

- Easily Manufactured
 - Quickly produced in response to emerging threats
 - Established and approved manufacturing procedures
- Favorable Safety Profile
 - Replication defective
 - Not transmissible
- No Pre-existing Vector Immunity
- Flexible Platform
 - Delivered by various methods
 - Easily combined to form multivalent vaccines



DNA VACCINES AT USAMRIID

Vaccines for Various Biodefense Threats

- Alphavirus
 - Venezuelan equine encephalitis virus (VEEV)
 - Eastern equine encephalitis virus (EEEV)
 - Western equine encephalitis virus (WEEV)
- Arenavirus
 - Lassa virus (LASV)
- Bacillus anthracis*
- Bunyavirus
 - Hantaan virus (HTNV)
 - Puumala virus (PUUV)
 - Rift Valley fever virus (RVFV)
 - Crimean Congo hemorrhagic fever virus (CCHFV)
- Filovirus
 - Ebola virus (EBOV)
 - Marburg virus (MARV)
- Flavivirus
 - Tick-borne encephalitis virus (TBEV)
- Poxvirus
 - Smallpox virus (VARV)
 - Monkeypox virus (MPXV)
 - Vaccinia virus (VACV)



DNA VACCINES AT USAMRIID

Multivalent DNA Vaccine Combinations

- Combination of Genes from One Pathogen
 - Poxvirus
- Combination of Genes from Diverse Pathogens
 - VEEV, EBOV, MARV, *B. anthracis*
 - TBEV, HNTV, RVFV, CCHFV
- Combination of Genes from Similar Pathogens
 - VEEV, EEEV, WEEV
 - HTNV, PUUV



DELIVERY OF DNA VACCINES

Technologies Evaluated at USAMRIID

- Particle-Mediated Epidermal Delivery (Gene gun)
- Skin Dermabrasion
- Microneedle Injection
- Transcutaneous Chemical
- Liquid Jet Injection
- Cationic Lipid
- Electroporation



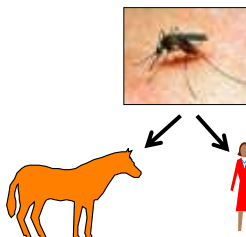
ENCEPHALITIC ALPHAVIRUSES

Venezuelan Equine Encephalitis Virus (VEEV)

Eastern Equine Encephalitis Virus (EEEV)

Western Equine Encephalitis Virus (WEEV)

- Disease in Humans
 - Fever, headache, myalgia, occasional encephalitis
- Case Fatality Rates
 - VEEV 1%
 - EEEV 30-70%
 - WEEV 8-15%
- Category B Select Agents
 - Ease of production
 - Considerable stability
 - High infectivity in aerosols



ENCEPHALITIC ALPHAVIRUSES

Existing IND Vaccines

Live-attenuated VEEV Vaccine (TC-83)

- Durable protective immune response
- High non-response rate (~20%)
- Reactogenic (~25%)
- Some reversion to virulence



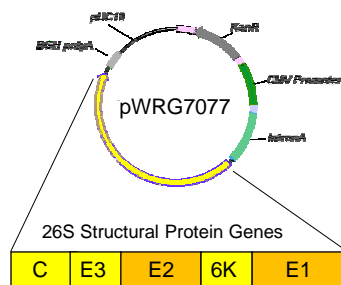
Formalin-inactivated VEEV, EEEV, WEEV Vaccines

- Well-tolerated
- Require frequent boosting
- Poor aerosol protection

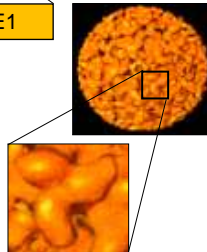


ALPHAVIRUS DNA VACCINES

Administration by Gene Gun

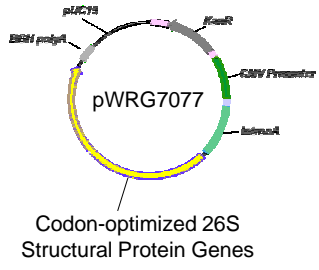


PowderMed XR-1



ALPHAVIRUS DNA VACCINES

Electroporation of Optimized Constructs

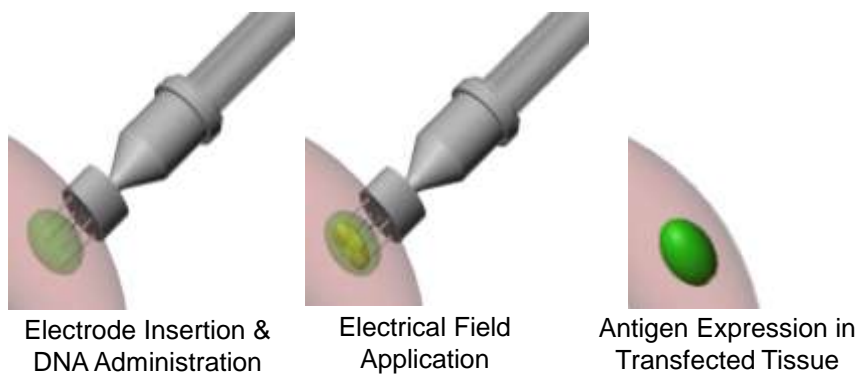


Ichor TriGrid™ Delivery System (TDS) EP Technology



ELECTROPORATION DELIVERY OF DNA

Increased Plasmid Uptake and Antigen Expression

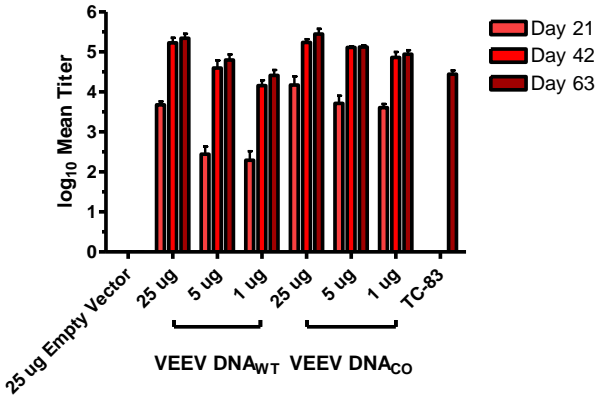


VEEV DNA IMMUNOGENICITY

Total IgG Antibodies by ELISA



N = 6

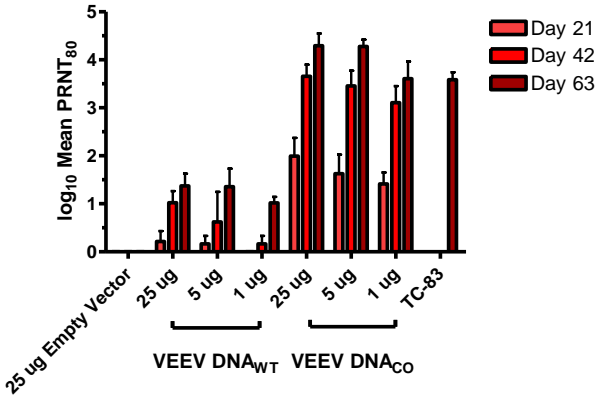


VEEV DNA IMMUNOGENICITY

Neutralizing Antibodies by PRNT



N = 6

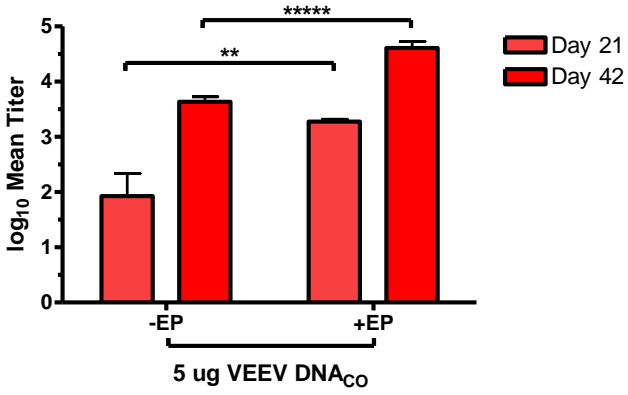


VEEV DNA IMMUNOGENICITY

Total IgG Antibodies by ELISA



N = 6



p < 0.01, **p < 0.0001

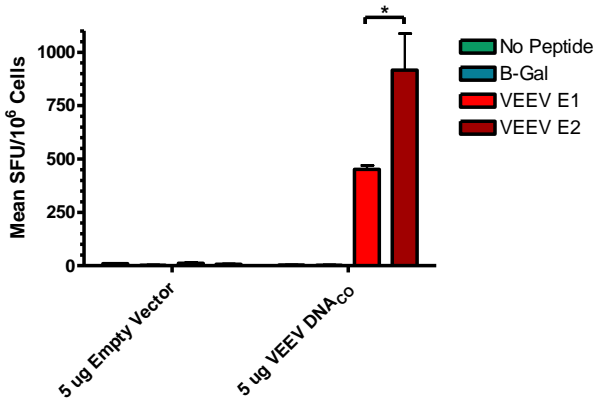


VEEV DNA IMMUNOGENICITY

Cellular Responses by IFN-γ ELISPOT



N = 6



*p < 0.05

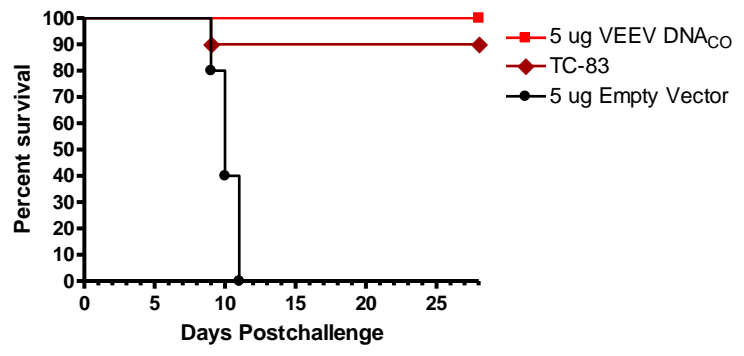


VEEV DNA PROTECTION

Aerosol VEEV Challenge Survival



N = 10



Aerosol challenge: $\geq 1,000$ LD₅₀

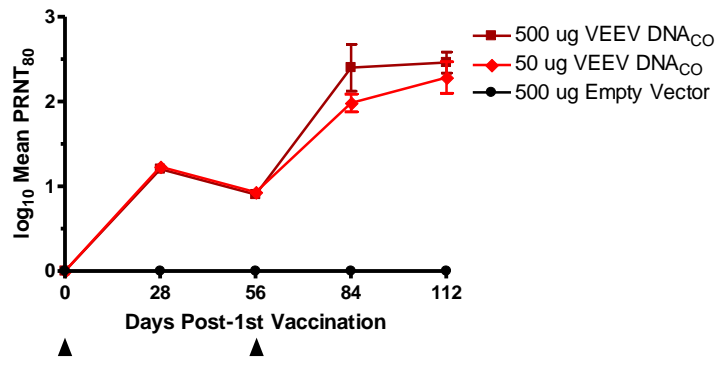


VEEV DNA IMMUNOGENICITY

Neutralizing Antibody Responses by PRNT



N = 4

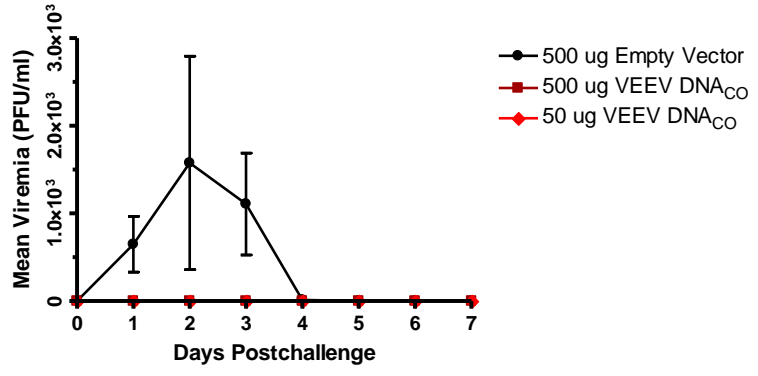


VEEV AEROSOL CHALLENGE

Serum Viremia by Plaque Assay



N = 4



Aerosol challenge: ~300 ED₅₀

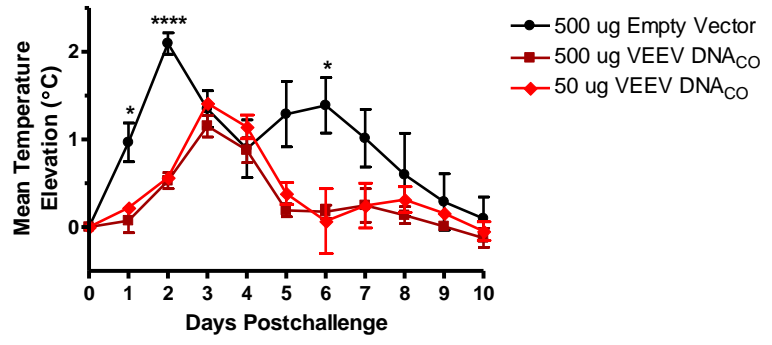


VEEV AEROSOL CHALLENGE

Fever Responses by Telemetry



N = 4



Aerosol challenge: ~300 ED₅₀
*p < 0.05, ****p < 0.001

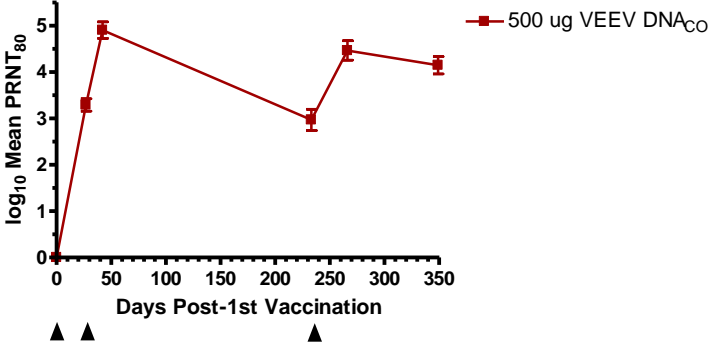


VEEV DNA IN RABBITS

Neutralizing Antibody Responses by PRNT



N = 5

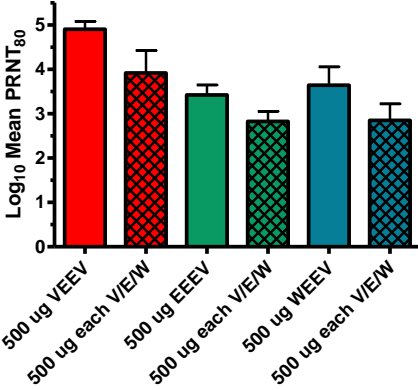


COMBINED ALPHAVIRUS DNA

Neutralizing Antibody Responses by PRNT



N = 5



ALPHAVIRUS DNA VACCINES

Summary and Conclusions

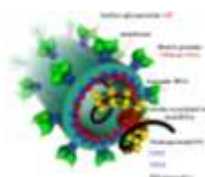
- Codon-optimized Alphavirus DNA vaccines are potent when delivered by electroporation.
 - Robust humoral and cellular immune responses
 - Durable neutralizing antibody responses
 - Potential to achieve protective immune responses at low doses and with few vaccinations
- Immune responses to codon-optimized Alphavirus DNA vaccines not significantly altered when delivered by electroporation as a trivalent combination.



FILOVIRUS VIRAL HEMORRHAGIC FEVERS

Ebola Virus (EBOV) and Marburg Virus (MARV)

- Negative-sense, single-stranded RNA viruses
- Epidemiology
 - Transmission by close contact (blood or body fluids)
 - Natural host unknown (suspected fruit bats)
- Clinical Features
 - Incubation period: 4-21 days
 - Abrupt onset of nonspecific symptoms
 - Impaired liver function
 - Bleeding and dysregulated coagulation (clotting)
 - Death/shock 6-9 days after onset
 - High case fatality rates (40-90%)
- Category A Select Agents
- Ebola virus species
 - Zaire
 - Sudan
 - Ivory Coast
 - Reston
 - Bundibugyo



MULTI-EPITOPE VACCINES

Genome-Derived Epitope-Driven Approach

- ❑ Highly accurate tools available for *in silico* identification of Class I/II T-cell epitopes
- ❑ Advantages to this approach for vaccine development:
 - ❑ **Effective:** Focus immune response on epitopes essential for protection
 - ❑ **Safe:** Elimination of epitopes against conserved “self” antigens
 - ❑ **Broad:** Selection of epitopes conserved across multiple strains or subtypes
 - ❑ **Fast:** Protective vaccines developed in 24 months



MULTI-EPITOPE VACCINES

EpiMatrix T-cell Epitope Predictions

- ❑ Input Sequences:
 - ❑ Zaire Ebola Virus (ZEBOV):
 - Glycoprotein (GP)
 - Nucleocapsid protein (NP)
 - ❑ Sudan Ebola Virus (SEBOV):
 - Glycoprotein (GP)
 - Nucleocapsid protein (NP)
 - ❑ Venezuelan equine encephalitis virus Subtype IAB (Strain Trinidad donkey)
 - 26S structural gene ORF



MULTI-EPITOPE VACCINES

EpiMatrix T-cell Epitope Predictions

Frame Start	AA Sequence	Frame Stop	Hydrophobicity	EB01*0301 Z-Score	EB01*0301 Z-Score	EB01*0401 Z-Score	EB01*0701 Z-Score	EB01*0801 Z-Score	EB01*1101 Z-Score	EB01*1301 Z-Score	EB01*1501 Z-Score	Hits
16	EEFFVWYI	24	37								1.85	0
17	EEFFVWYII	25	40									0
18	EEFFVWYIIL	26	50									0
19	EEFFVWYIILP	27	3.14			1.98	2.21		1.29	1.88		3
20	EEFFVWYIILRP	28	2.44			2.82	2.36	1.49	2.32	1.53	1.54	3
21	EEFFVWYIILRPD	29	1.63									0
22	EEFFVWYIILRPDQ	30	1.13	1.50		1.34	2.85		1.50			1
23	EEFFVWYIILRPDQK	31	1.54				1.43		1.80			1
24	EEFFVWYIILRPDQKQ	32	39	1.57	1.57	1.25		2.88	1.60	2.71	1.35	4
25	EEFFVWYIILRPDQKQK	33	39	2.1	2.94	1.29	1.83	1.51	1.93	2.17	2.11	6
26	EEFFVWYIILRPDQKQKQ	34	31									0
27	EEFFVWYIILRPDQKQKQK	35	31	2.79	1.68	2.26	1.26	2.17	1.45	2.15	2.03	6
28	EEFFVWYIILRPDQKQKQKQ	36	-01									0
29	EEFFVWYIILRPDQKQKQKQK	37	17		1.25		1.57				1.30	0
30	EEFFVWYIILRPDQKQKQKQKQ	38	39									0

- Epivax *in silico* class II binding prediction
- Cluster with multiple predicted epitopes in ZEBOV GP



MULTI-EPITOPE VACCINES

HLA Class II Epitope Selections

Input Sequence	Cluster Address (w FLANKS)	Cluster Sequence	Hydro-phobicity	EpiMatrix HITS (w/o FLANKS)	EpiMatrix CLUSTER SCORE (w/o FLANKS)	EpiBars? (#)
EBOLA-SUDAN-GP	180 - 196	VIAFLILAKPKETFLQS	0.94	12	24.01	Yes (2)
EBOLA-SUDAN-GP	230 - 245	STLTKINNNTFVLLD	0.31	14	29.69	Yes (2)
EBOLA-SUDAN-GP	249 - 265	TPQFLQLNDTHLHQQ	-0.52	14	24.76	Yes (2)
EBOLA-SUDAN-GP	402 - 421	GTGLSSQILSSSPPTMA*SP	-0.02	13	38.76	Yes (2)
EBOLA-SUDAN-GP	544 - 559	TEGLMHNQNALVCSLR	-0.09	11	30.29	Yes (1)
EBOLA-SUDAN-GP	566 - 583	TOALQLFLRATTELRTYT	-0.15	12	22.54	Yes (2)
EBOLA-SUDAN-GP	579 - 595	LRTYFILNRKAIIDFLR	0.1	11	21.15	Yes (1)
EBOLA-SUDAN-NP	18 - 35	DLDYHKILTAGLSVQQGI	0.09	11	19.49	Yes (1)
EBOLA-SUDAN-NP	70 - 87	ADSFLLLLCLHAYQGDH	0.29	9	16.14	No
EBOLA-SUDAN-NP	195 - 212	GHAMVIFRLMRTNFKLF	0.79	13	31.49	Yes (1)
EBOLA-SUDAN-NP	293 - 311	YAPFARLLNSGVNLEHG	-0.02	14	26.48	Yes (2)
EBOLA-SUDAN-NP	649 - 664	EETTYHLKIQGFPEA	-0.93	9	17.81	Yes (2)
EBOLA-ZAIRE95-GP	32 - 49	SIFLGVHNSLQVSDVD	0.38	12	38.96	Yes (1)
EBOLA-ZAIRE95-GP	85 - 99	RHWGFRSGVPPKVVNY	-0.53	8	15.39	Yes (1)
EBOLA-ZAIRE95-GP	238 - 252	NLTYVQLERFTT*GF	-0.41	6	13.81	Yes (1)
EBOLA-ZAIRE95-GP	249 - 265	TPQFLQLNETYTSQK	-0.32	14	27.14	Yes (2)
EBOLA-ZAIRE95-GP	336 - 352	TEQHKIMASENSAMVQ	-0.88	10	15.83	Yes (1)
EBOLA-ZAIRE95-NP	80 - 101	HHAYGGDYKFLFESGAVKYLEG	-0.55	15	22.77	Yes (2)
EBOLA-ZAIRE95-NP	273 - 292	VKN*EVNSFKALSLAK*HGE	-0.38	14	32.89	Yes (2)
EBOLA-ZAIRE95-NP	645 - 665	EHSFEEMYRHILRSQGFDAV	-0.76	15	22.23	Yes (3)
VEEV-26S	30 - 50	TDFFLAMGVQLTRSMANLTF	0.1	15	25.6	Yes (2)
VEEV-26S	375 - 390	HGGVYRLQTSQYGLD	-0.98	13	29.09	Yes (2)
VEEV-26S	429 - 444	GHWYFLLRARCPAGDSI	0.31	9	17.71	Yes (1)
VEEV-26S	718 - 734	ASTWLFCSRVAQLTPY	0.44	10	17.63	Yes (2)
VEEV-26S	1029 - 1045	NTNLVLRPKAGAHVP	-0.14	11	20.43	Yes (1)



MULTI-EPITOPE VACCINES

Summary and Conclusions

- ❑ Putative Class II T-cell epitopes in the structural proteins of ZEBOV, SEBOV, and VEEV predicted with EpiMatrix
- ❑ Predicted Class II epitopes validated by *in vitro* HLA binding assays
 - ❑ 74% of EpiMatrix predictions met or exceeded in binding assays
- ❑ Optimal order of selected Class II epitopes determined with VaxCad
- ❑ Final Class II multi-epitope DNA vaccine constructed
- ❑ Currently initiating studies to evaluate immunogenicity and protective efficacy of Class II construct in HLA transgenic mice



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❑ Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.