



Attenuation of the Horsepox Virus Platform as a Vaccine for Mpox/Smallpox (TNX-801)

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World Vaccine Congress: April 3rd, 2024





Cautionary Note on Forward-Looking Statements

Certain statements in this presentation regarding strategic plans, expectations and objectives for future operations or results are “forward-looking statements” as defined by the Private Securities Litigation Reform Act of 1995. These statements may be identified by the use of forward-looking words such as “anticipate,” “believe,” “forecast,” “estimate” and “intend,” among others. These forward-looking statements are based on Tonix’s current expectations and actual results could differ materially. There are a number of factors that could cause actual events to differ materially from those indicated by such forward-looking statements. These factors include, but are not limited to, the risks related to failure to obtain FDA clearances or approvals and noncompliance with FDA regulations; delays and uncertainties caused by the global COVID-19 pandemic; risks related to the timing and progress of clinical development of our product candidates; our need for additional financing; uncertainties of patent protection and litigation; uncertainties of government or third party payor reimbursement; limited research and development efforts and dependence upon third parties; and substantial competition. As with any pharmaceutical under development, there are significant risks in the development, regulatory approval and commercialization of new products. The forward-looking statements in this presentation are made as of the date of this presentation, even if subsequently made available by Tonix on its website or otherwise. Tonix does not undertake an obligation to update or revise any forward-looking statement, except as required by law. Investors should read the risk factors set forth in the Annual Report on Form 10-K for the year ended December 31, 2023, as filed with the Securities and Exchange Commission (the “SEC”) on April 1, 2024, and periodic reports and current reports filed with the SEC on or after the date thereof. All of Tonix's forward-looking statements are expressly qualified by all such risk factors and other cautionary statements.

*TNX-801 has not been approved for any indication.



Outline of Talk

- 1) Background
- 2) TNX-801 attenuation *in vitro*
- 3) TNX-801 attenuation *in vivo*
- 4) TNX-801 immunogenicity and efficacy in NHP model

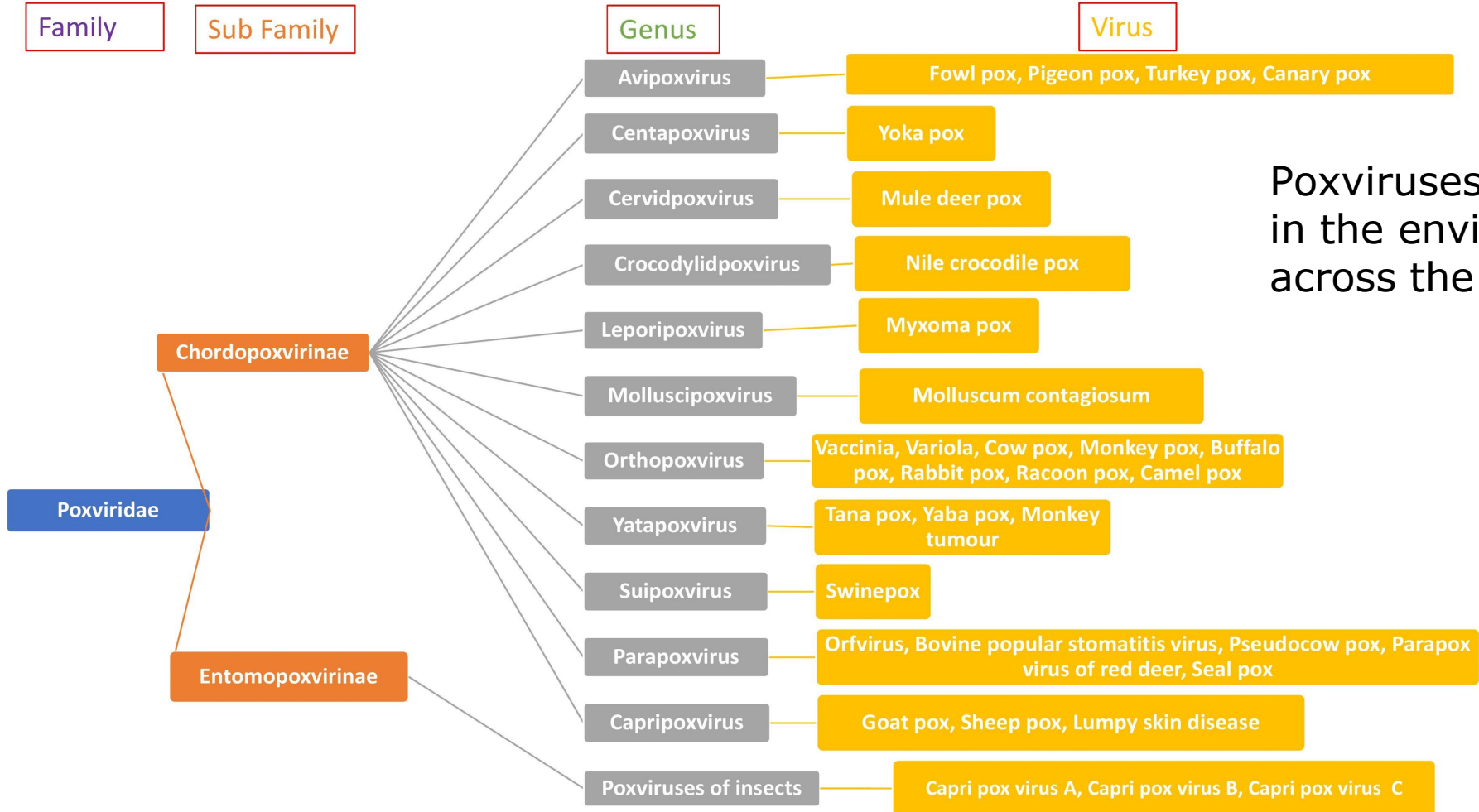


Background: Poxviruses

- Family: ***Poxviridae***
- Two subfamilies:
 - 1) *Chordopoxvirinae*
 - 2) *Entomopoxvirinae*
- 22 Genera
- Genome: Double stranded DNA, ~128-456 kb size
- Virions: enveloped, brick-shaped, ~220 to 450 nm long × 140 to 260 nm wide × 140 to 260 nm thick
- Infect vertebrate and invertebrate hosts



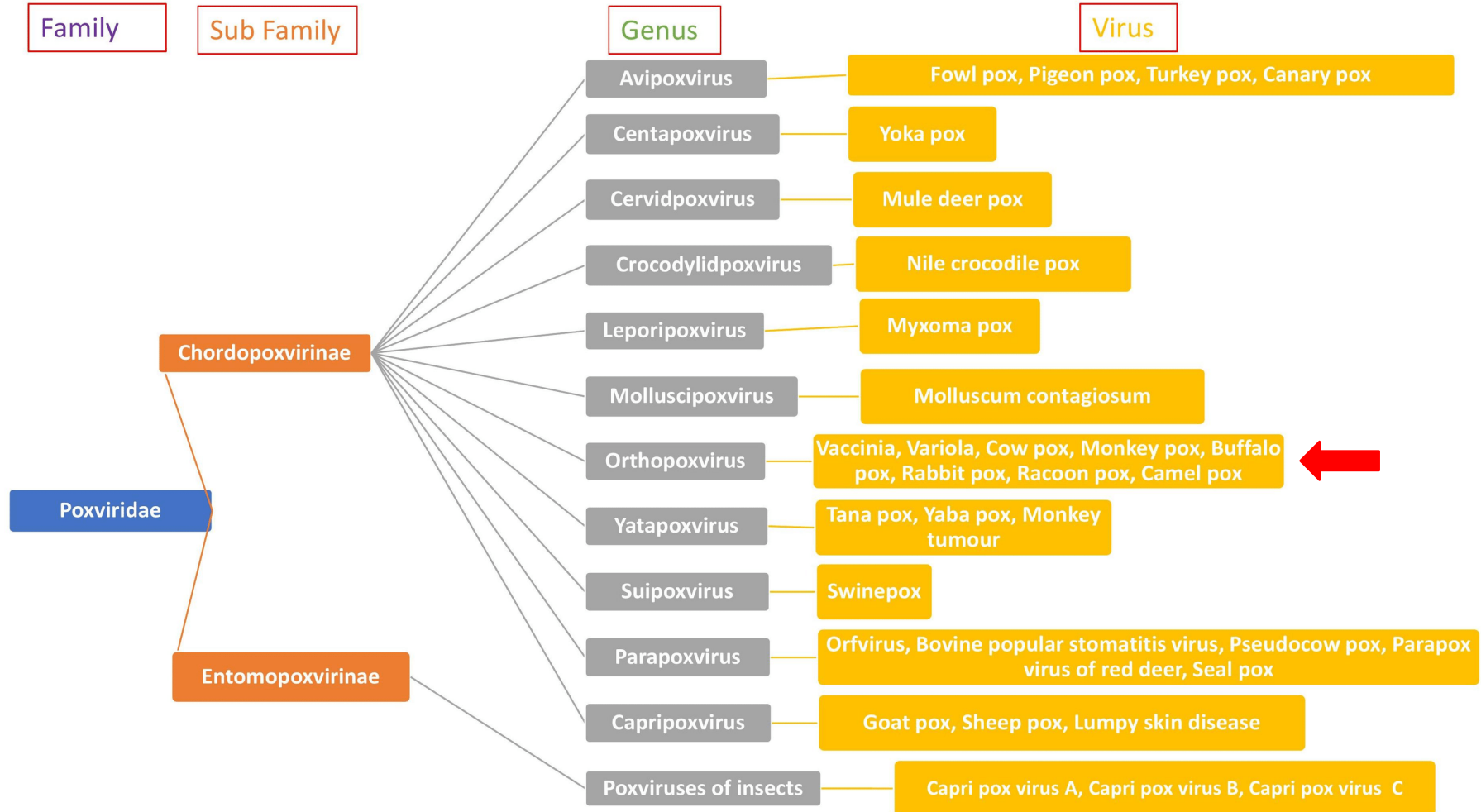
Poxviruses



Poxviruses are ubiquitous in the environment across the globe



Orthopox Viruses





Monkeypox Virus

- Endemic in Central and West Africa
- Two Clades:
 - 1) Clade I (DRC)
 - 2) Clade IIa (West Africa) and IIb (Nigeria)
- Human Case Fatality Rate:
 - Clade I – ~11%
 - Clade IIa – ~3%
 - Clade IIb – <0.1%
- Clade IIb – 2022 Outbreak
 - 116 Countries
 - ~93,000 Confirmed Cases

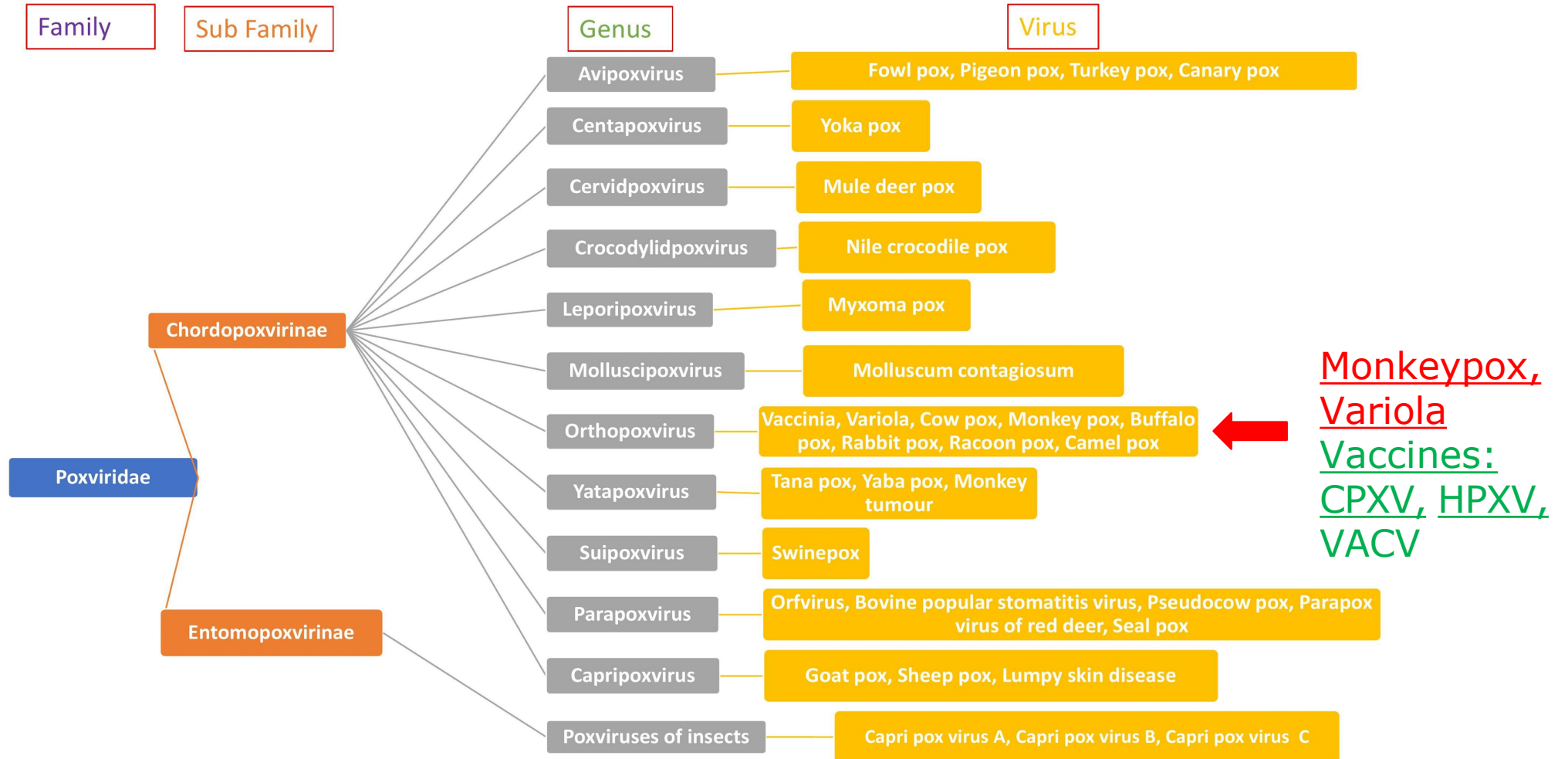


Variola Virus (Smallpox)

- Oldest written record – ~3,500 years
- Oldest sequences – ~1,400 years
- Human Case Fatality Rate: ~ 30%
- 20th century – ~250 to 500 million deaths
- Eradication: 1980

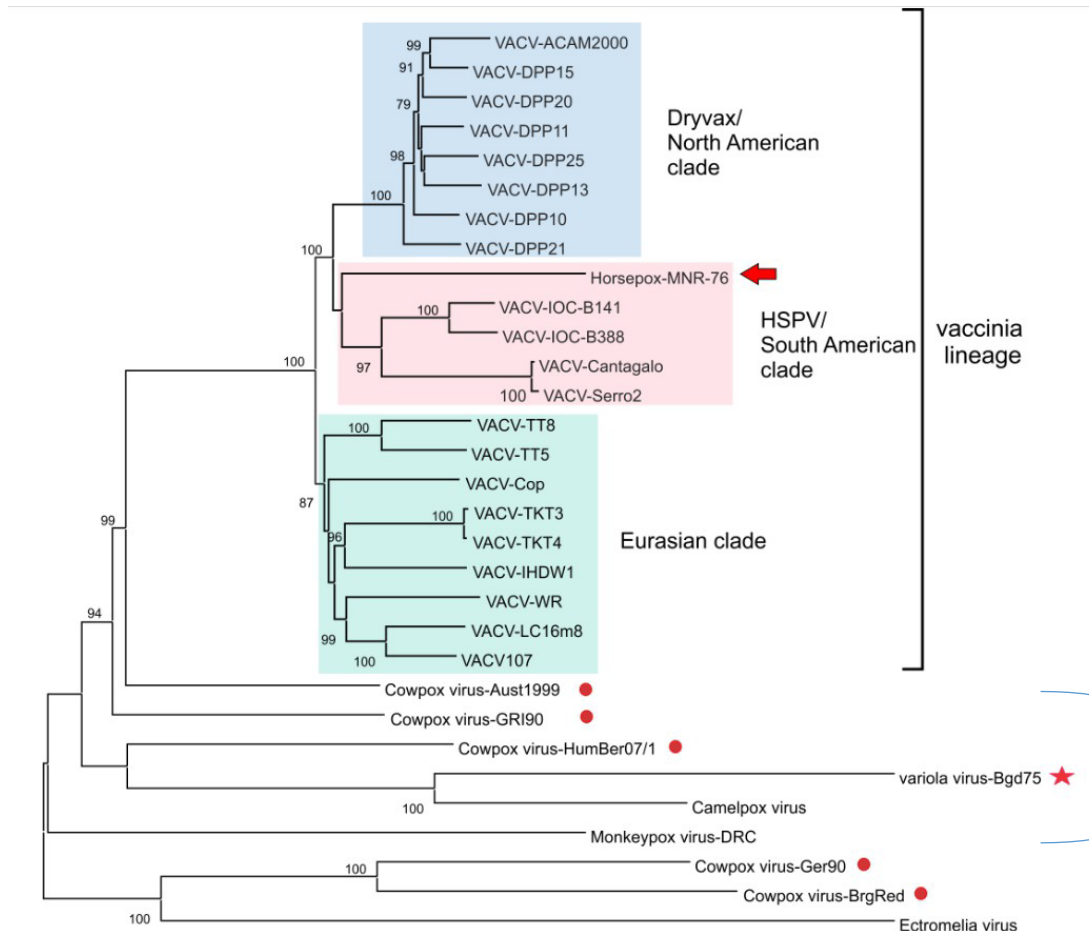


Orthopox Viruses





Phylogenetic Tree of Genus *Orthopox*



Cowpox close orthopox relative to smallpox (Variola)
Conferred protection against Variola



In 1796, Edward Jenner Successfully Used Vaccination to Protect Against Smallpox

- Jenner observed milkmaids were protected from smallpox, reasoned that infection with an illness similar to smallpox but less deadly could protect one against smallpox
 - “Cowpox” was the name of a disease in cows that could transfer to humans and cause sores
 - Jenner “vaccinated” (from *vacca*, Latin for “cow”) a patient with pustule matter from “cowpox” sores on a milkmaid’s hands; that patient remained healthy when challenged with smallpox virus
- Jenner suspected that the agent causing cowpox, which he called **vaccinia** originated in horses and had been transferred from horses to cows’ udders by dirty hands

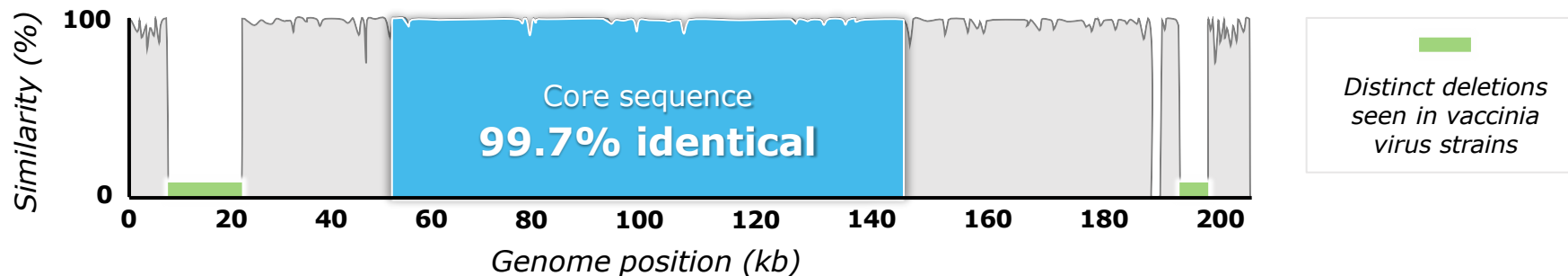




Equination, Use of Vaccines From Horses, Was Also Effective Against Smallpox

- Equination, the use of vaccines from horses (*equus* in Latin), was successfully used in parallel with vaccination in Europe¹
- Vaccine producers may have propagated stocks by periodically supplementing or refreshing them with horsepox²
- A 1902 smallpox vaccine (**Mulford**) – 99.7% identical to core viral sequence

Sequence Identity for the 1902 Mulford Vaccine Compared to HPVX³

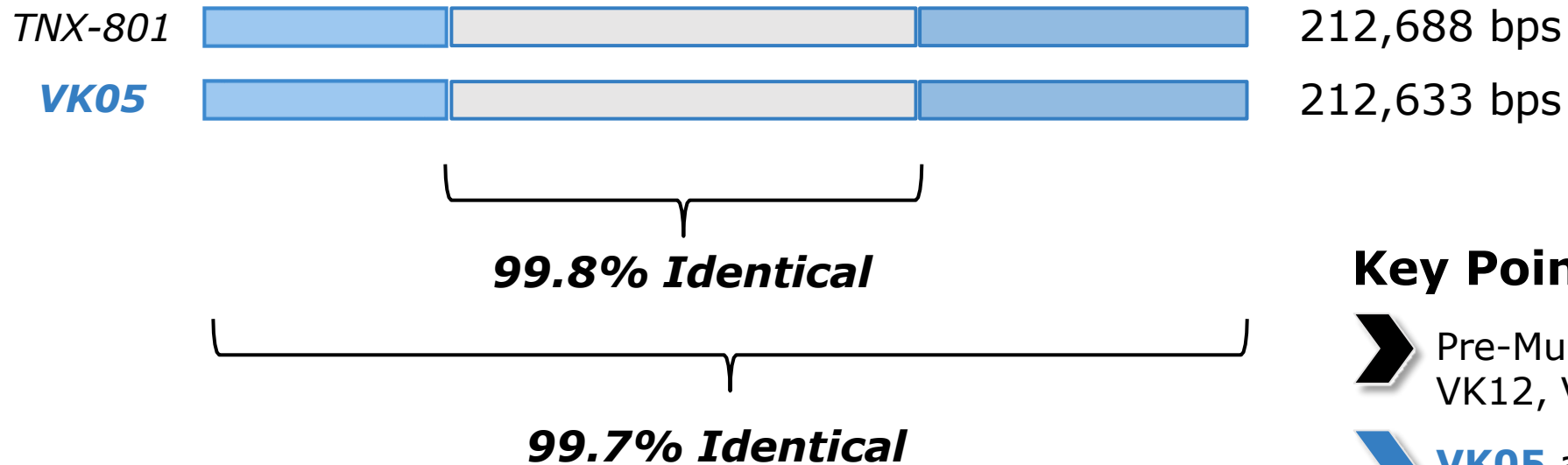


1. Esparza J, et al. *Vaccine*. 2017;35(52):7222-7230.
2. Esparza J, et al. *Vaccine*. 2020;38(30):4773-4779.
3. Schrick L, et al. *N Engl J Med*. 2017;377(15):1491-1492.



HPXV Was Used as Civil War-Era (1860s-1870s) Vaccine

VK05 has the highest identity to HPXV across the whole genome and represents a true HSPV strain



Key Points

- Pre-Mulford vaccines: VK05, VK12, VK02, VK08, and VK01
- **VK05** and **TNX-801** (HPXV) have colinear structural identity across their whole genome

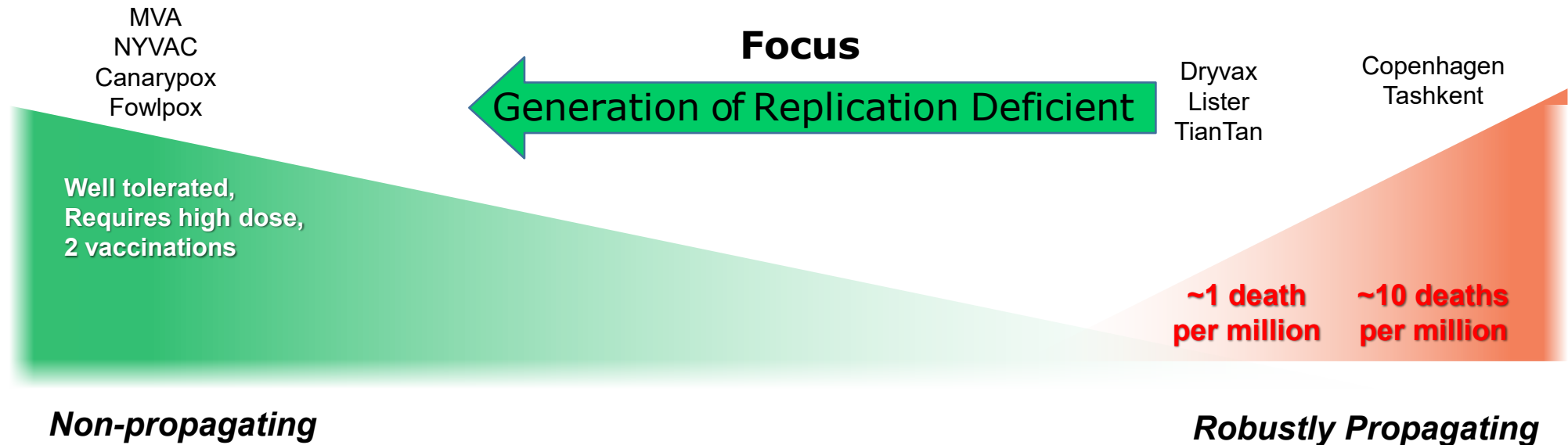


Smallpox Vaccines

- Vaccine: Cowpox origin
- Serial passaging: Humans, cows, and horses (143 years)
- Vaccine: Vaccinia Virus (1939) closely related to cowpox but serologically distinct
- Multiple Vaccinia virus-based vaccines developed
- Smallpox eradication

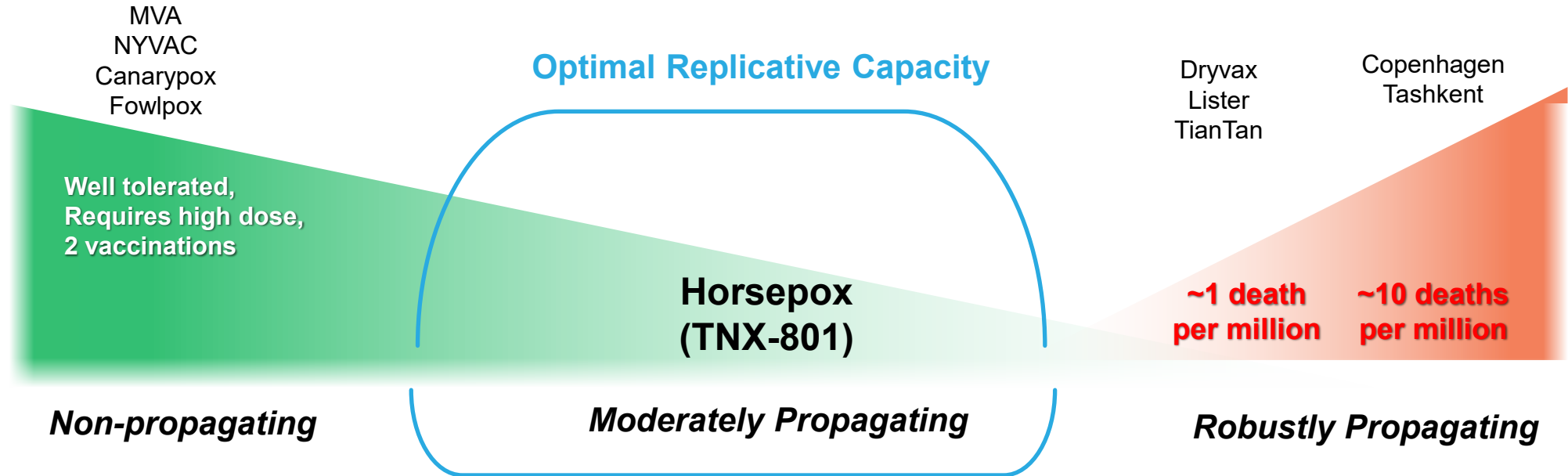


Balance of Tolerability and Reactogenicity for Pox-based Vaccines





Balance of Tolerability and Reactogenicity for Pox-based Vaccines



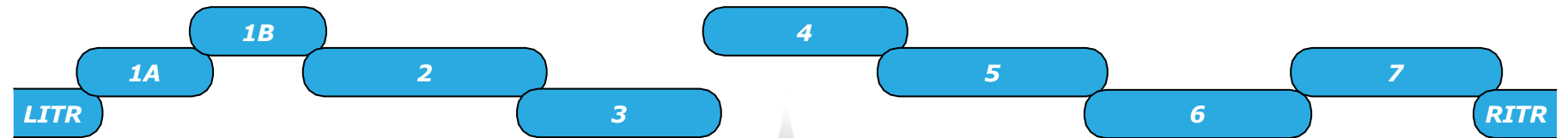


TNX-801 Core Genome Is Identical to the Published HPXV Strain MNR-76

MNR-76 genome (212,633 bp) *Genbank accession DQ792504*



MNR-76 genome fragments

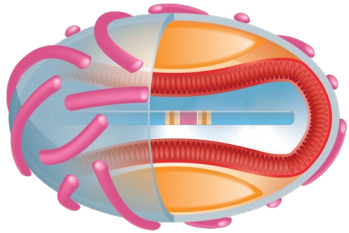


Ten overlapping genome fragments were assembled based on sequence homology to generate the TNX-801 genome^{1,2}

TNX-801 genome (212,811 bp) *Genbank accession KY349117*



The core genome of TNX-801 is identical to MNR-76¹



TNX-801

schPXV (Horsepox)
212,811 bp

1. Noyce RS, et al. *PLoS One*. 2018;13(1):e0188453.
2. Schrick L, et al. *N Eng J Med*. 2017;377(15):1491-1492.



2) TNX-801 attenuation *in vitro*

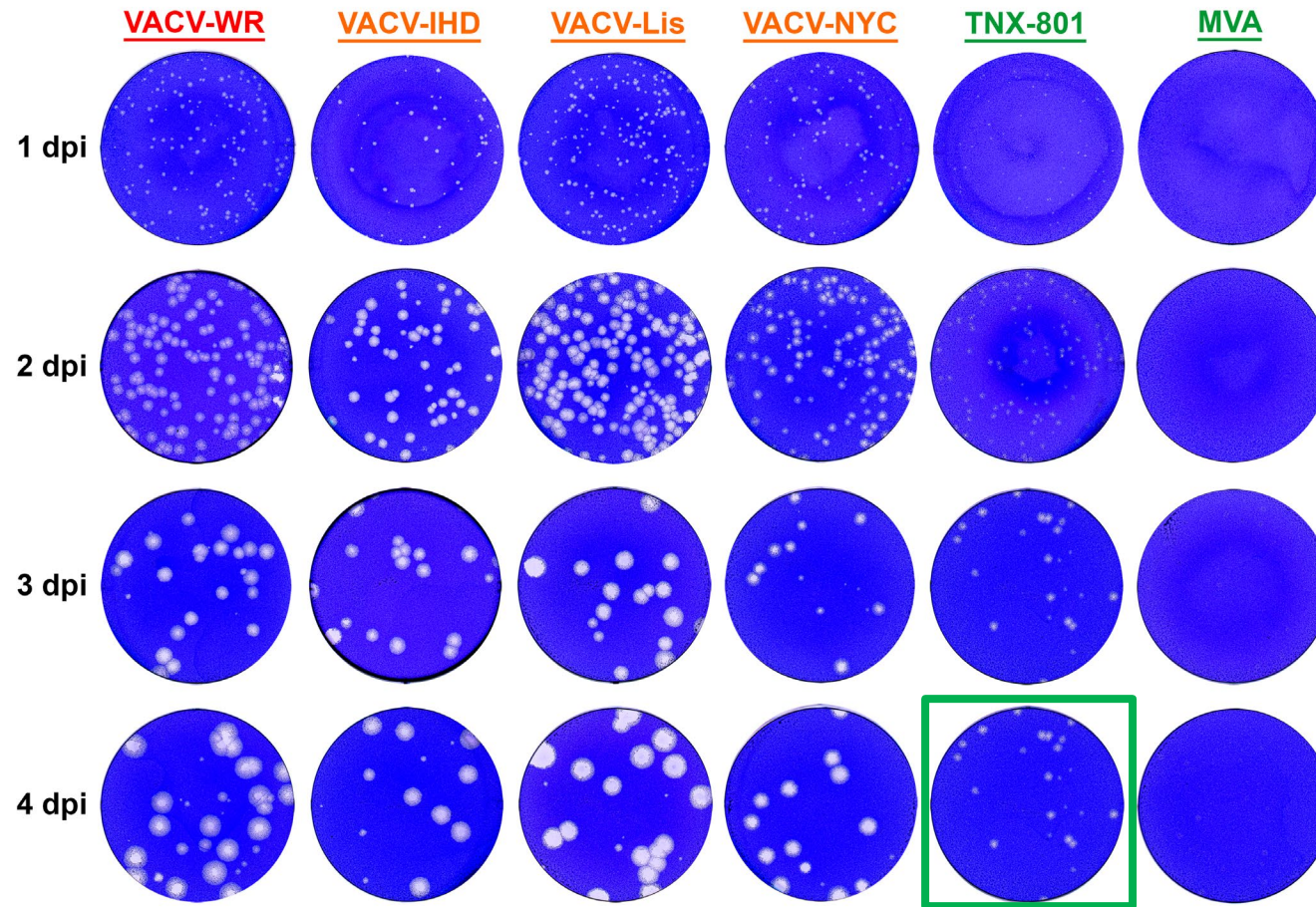


TNX-801 Characterization *in vitro*

- Investigate attenuation of TNX-801 *in vitro* relative to VACV strains
 - Positive Control: **VACV-Western Reserve (WR)**
 - Older vaccines used in smallpox eradication:
 - 1) VACV-International Health Department (IHD)
 - 2) VACV-Lister (Lis)
 - 3) VACV-New York City Board of Health (NYCBH)
 - New Vaccine: **MVA**
- 1) Plaque phenotype – BSC-40 and Vero-E6
- 2) Replication Kinetics
 - Immortalized non-human primate cell lines
 - Human primary cells from two main route of poxvirus transmission
 - Dermal and respiratory tracts

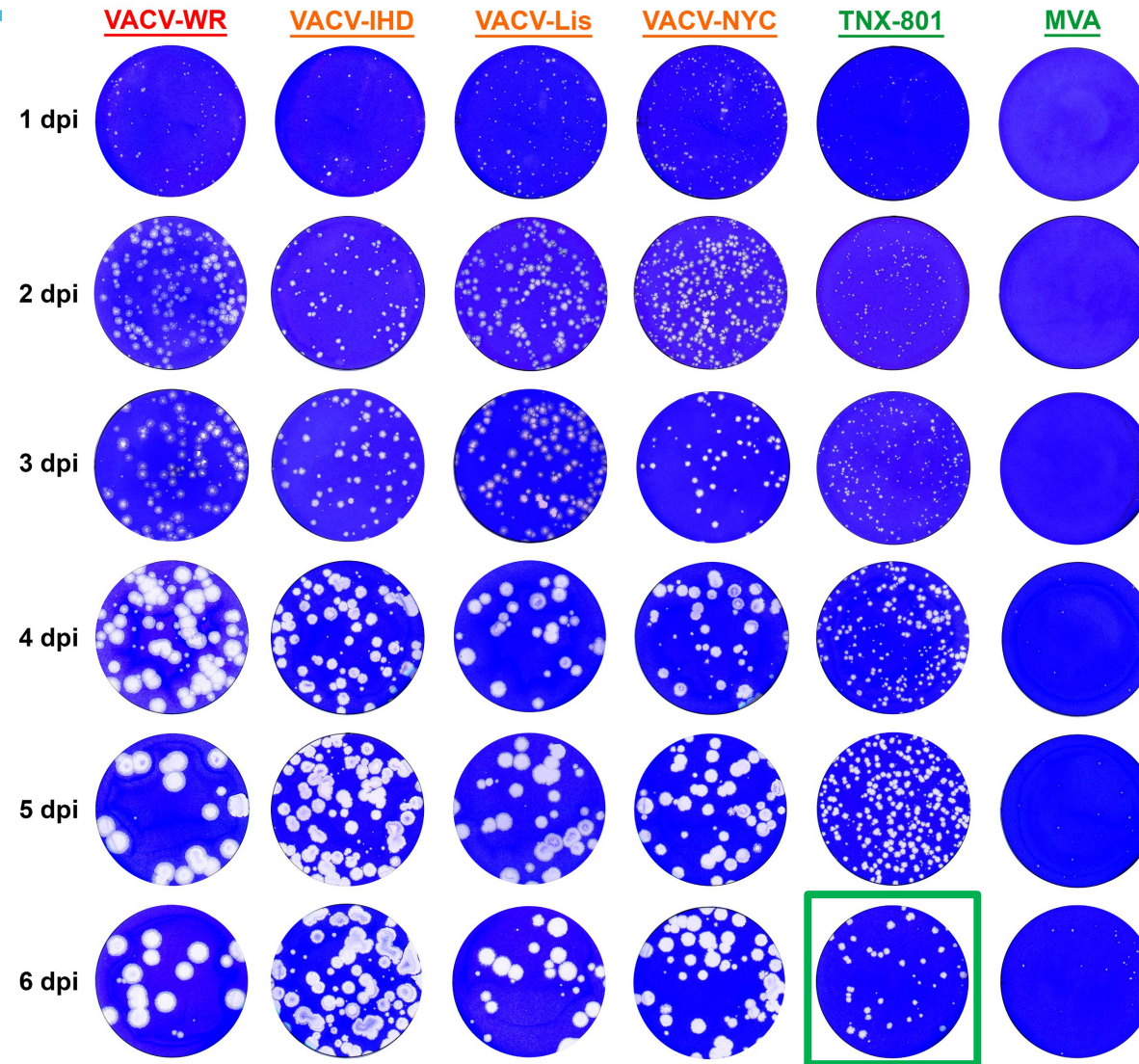


TNX-801: Small plaque phenotype in BSC-40 Cells





TNX-801: Small plaque phenotype in Vero-E6 Cells

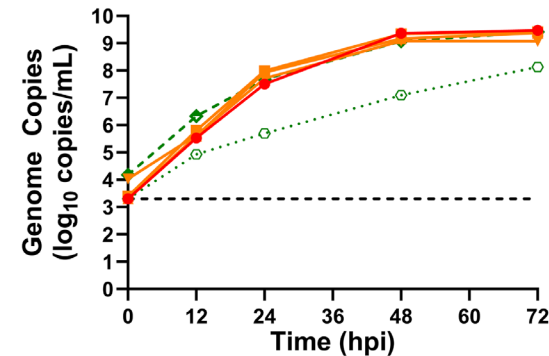
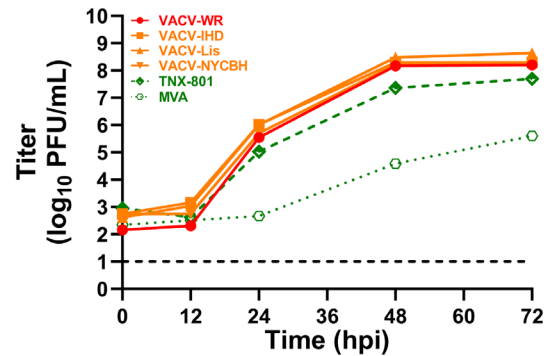


TNX-801: Replication in BSC-40 and Vero-E6

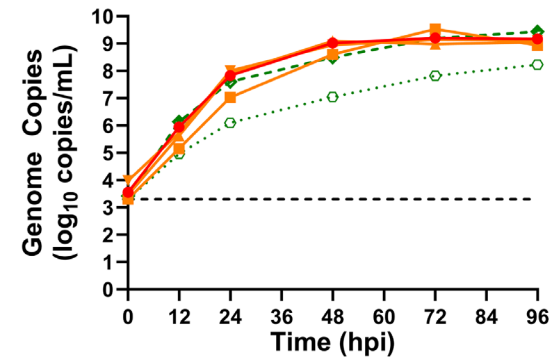
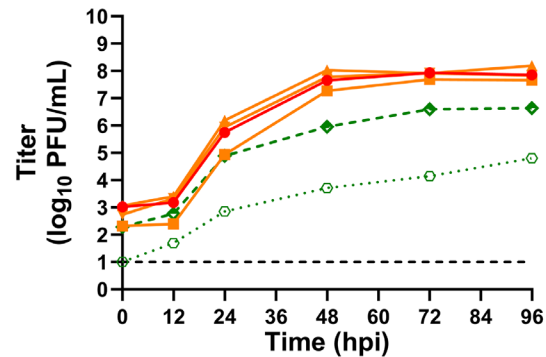
Plaque Assay

qPCR

BSC-40



Vero E6



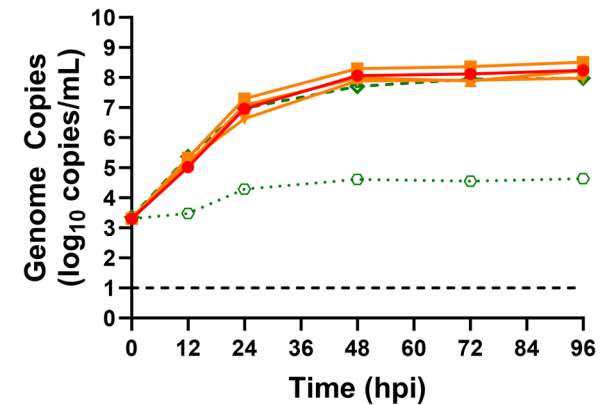
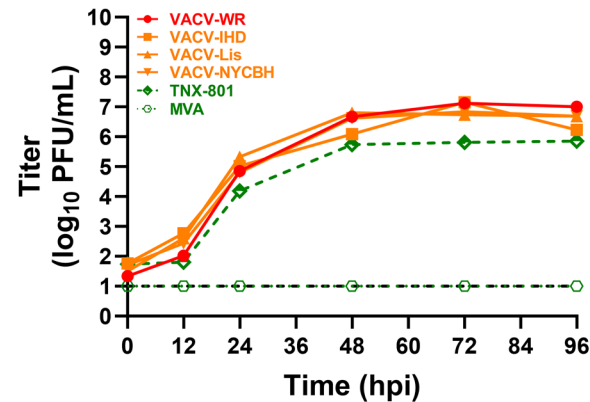
TNX-801: >10- to 100-fold more attenuated than VACV based vaccines

TNX-801: Replication in Primary Human Cells (Dermal Tract)

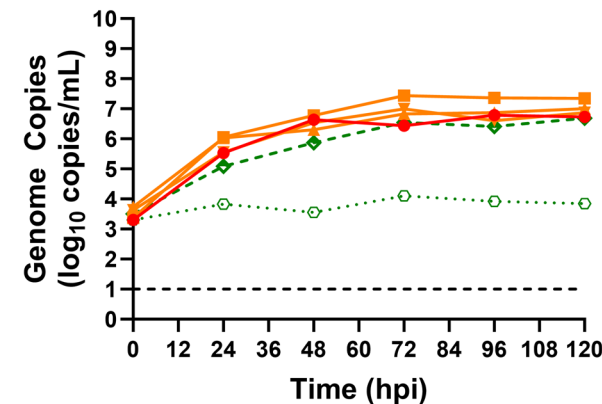
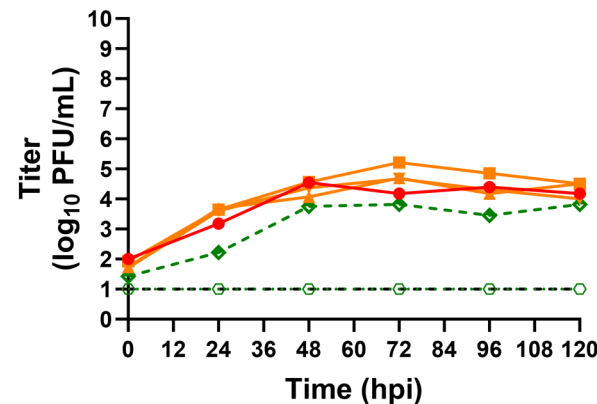
Plaque Assay

qPCR

Melanocytes



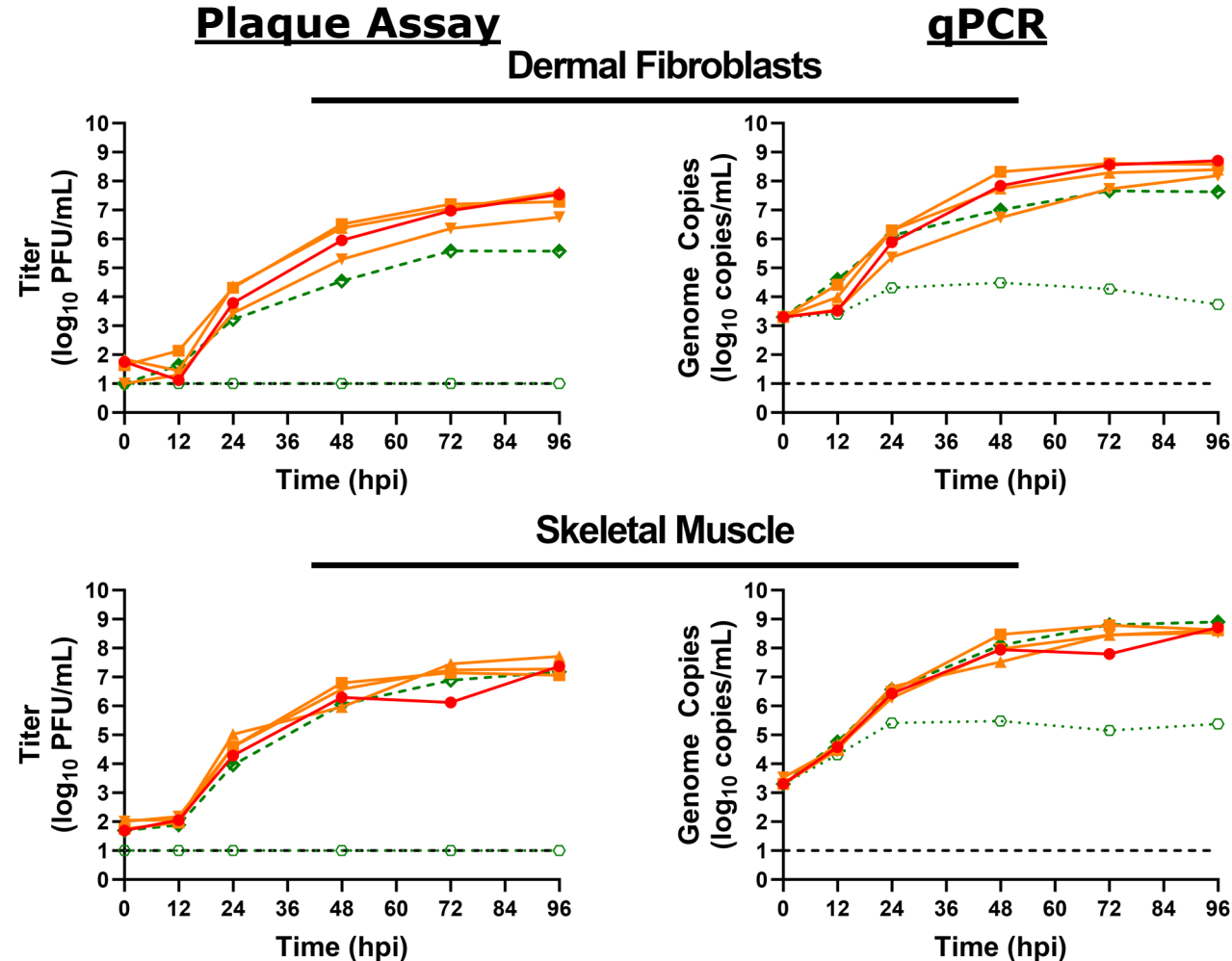
Keratinocytes



TNX-801: ~10- to 27-fold more attenuated than VACV based vaccines



TNX-801: Replication Kinetics in Primary Human Cells (Dermal Tract)



TNX-801: >5- to 119-fold more attenuated than VACV based vaccines

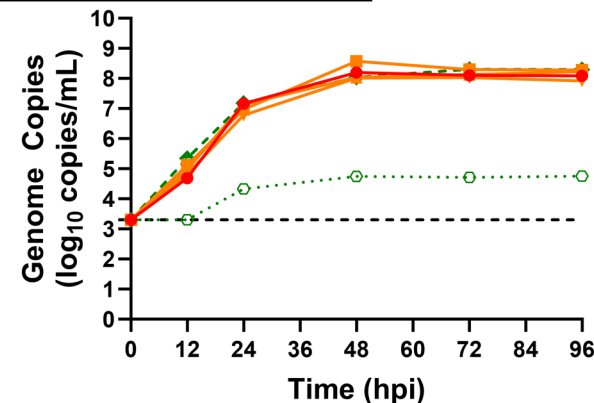
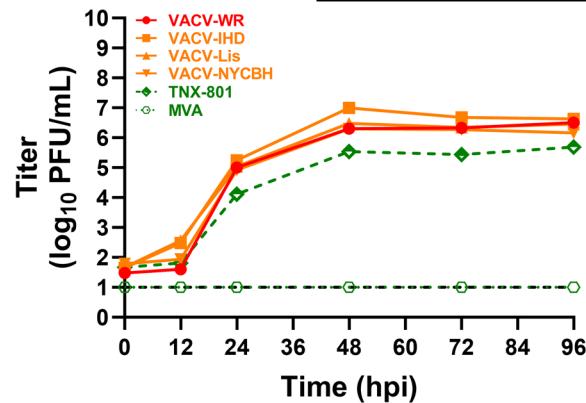


TNX-801: Replication Kinetics in Primary Human Cells (Respiratory Tract)

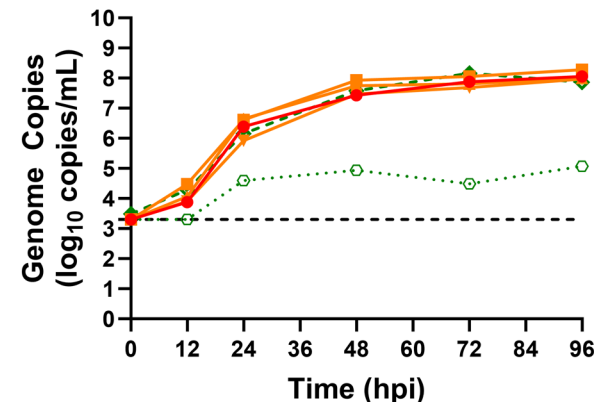
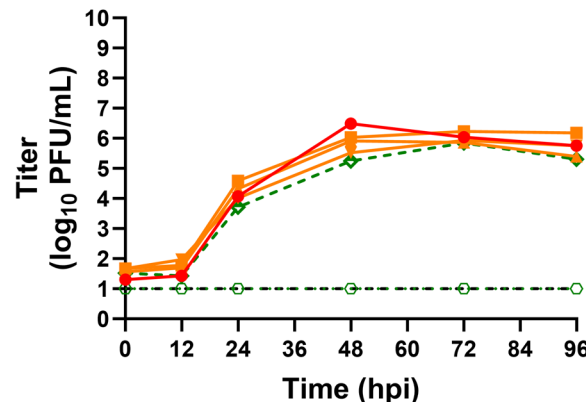
Plaque Assay

qPCR

B-T Epithelial



Small Airway Epithelial



TNX-801: ~20- to 30-fold more attenuated than VACV based vaccines

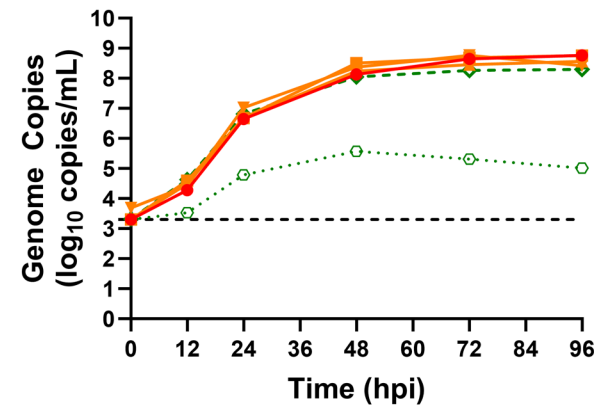
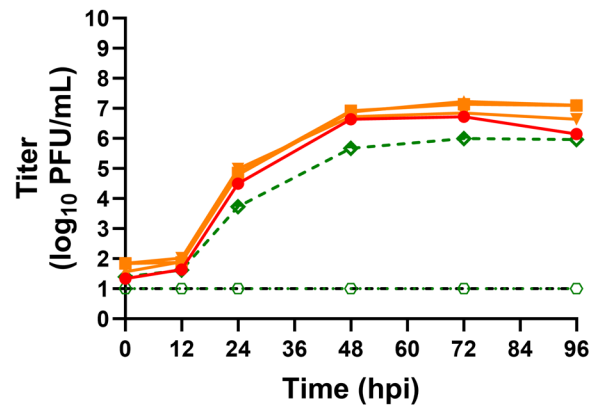


TNX-801: Replication Kinetics in Primary Human Cells (Respiratory Tract)

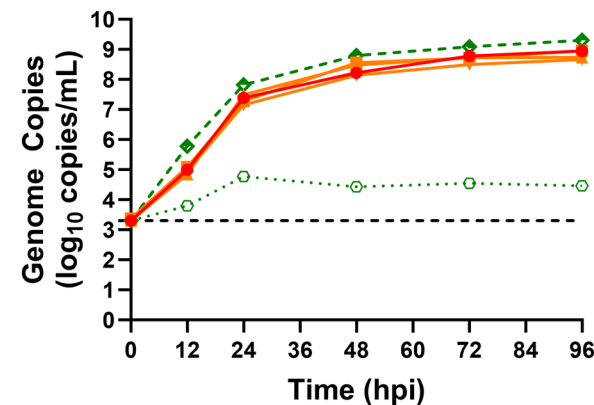
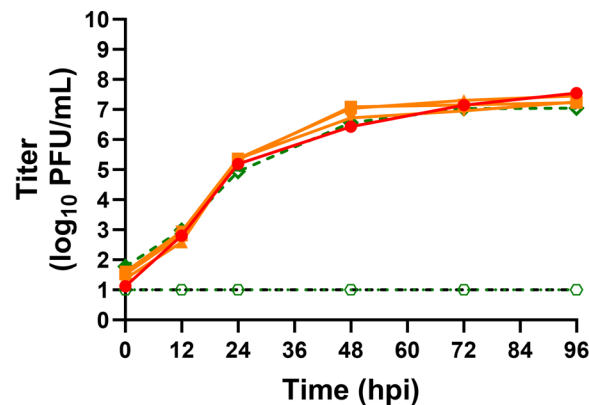
Plaque Assay

qPCR

Lung Fibroblasts



B-T Smooth Muscle



TNX-801: >3- to 30-fold more attenuated than VACV based vaccines



3) TNX-801 attenuation *in vivo*



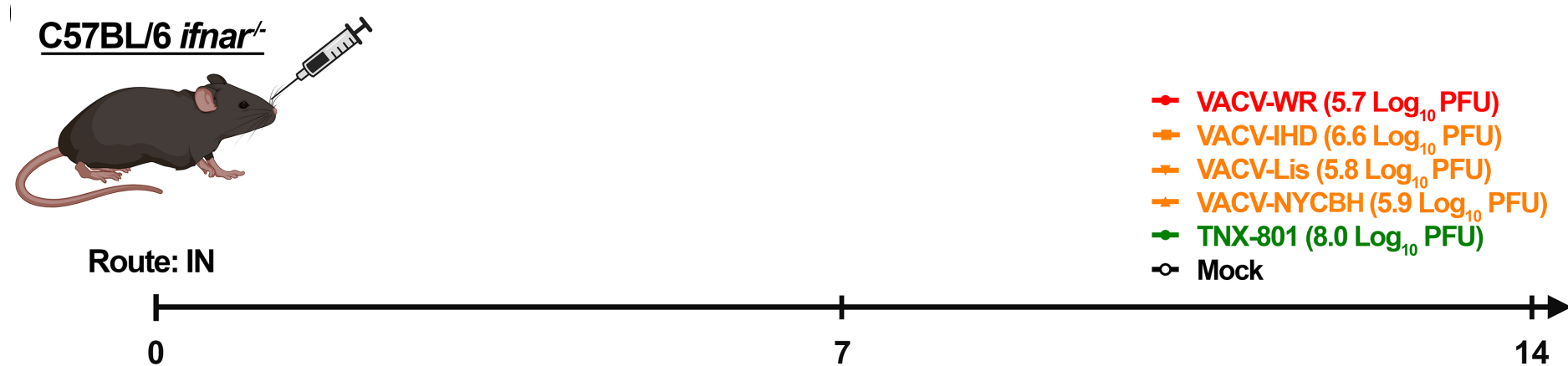
TNX-801 Characterization *in vivo*

- Investigate attenuation of TNX-801 *in vivo* relative to VACV based vaccines
 - Immunocompromised Mice (C57BL/6 *ifnar*^{-/-} and C57BL/6 *ifnar*^{-/-}/*ifngr*^{-/-})
 - Sensitive to virus infection
 - Positive Control: VACV-WR
 - Older vaccines: VACV-IHD, VACV-Lis, VACV-NYCBH
 - TNX-801
 - Route: Intranasal

- Parameters measured:
 - 1) Clinical Score
 - 2) Temperature
 - 3) Weight loss
 - 4) Survival



TNX-801 Characterization C57BL/6 *ifnar*^{-/-}

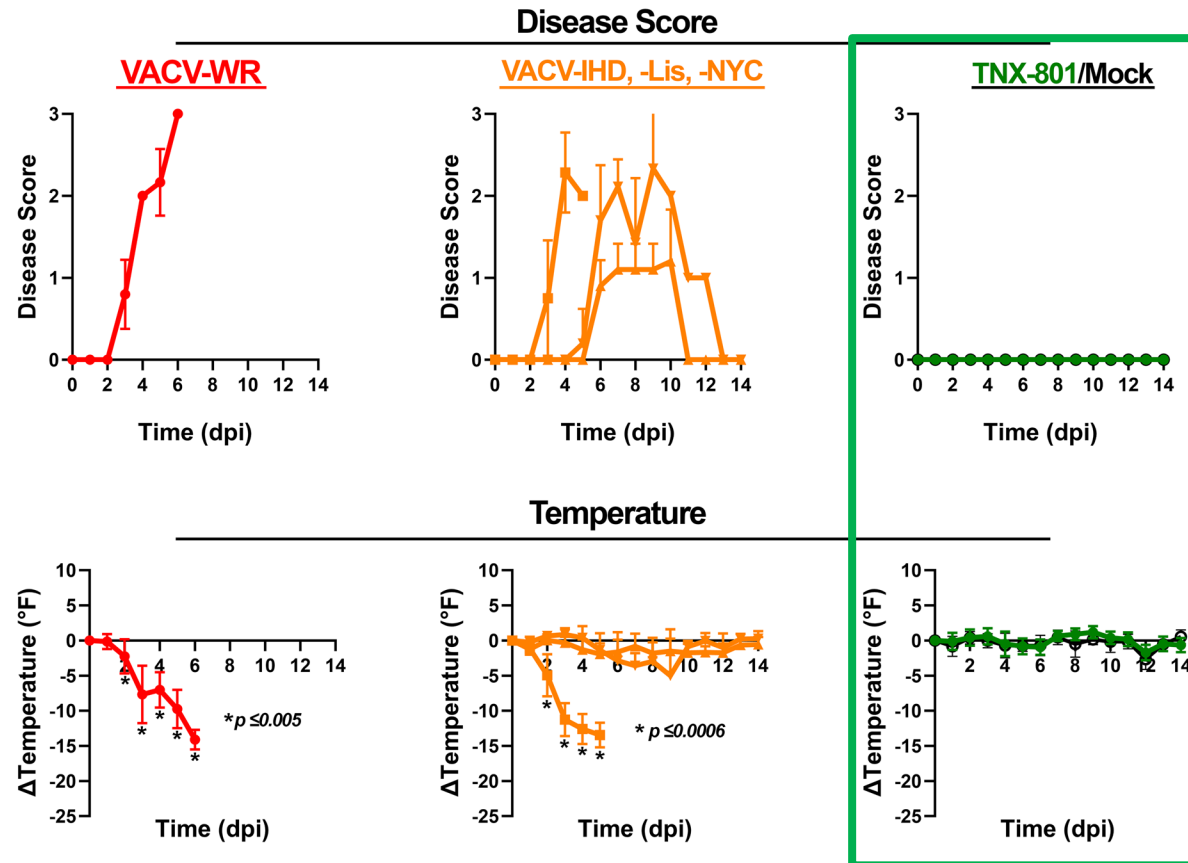


➤ **Parameters measured:**

- 1) Clinical Score
- 2) Temperature
- 3) Weight loss
- 4) Survival



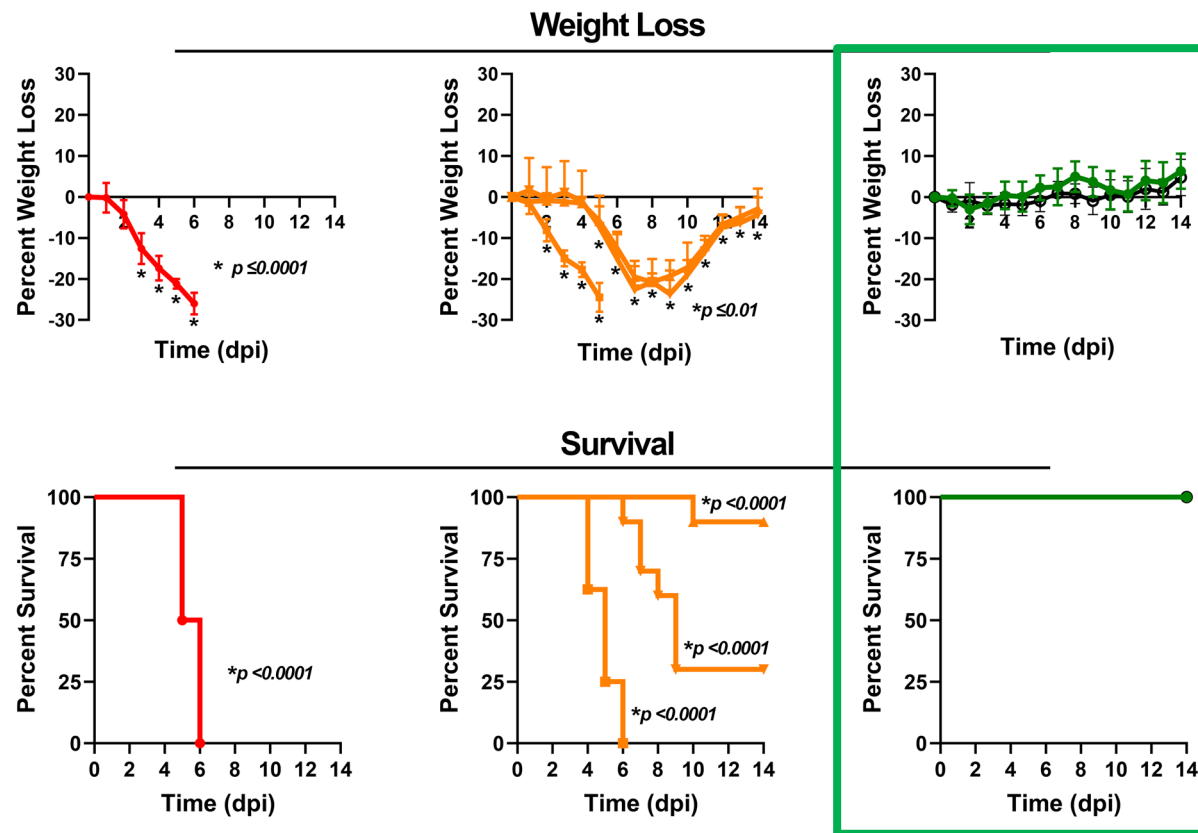
TNX-801 Infection of C57BL/6 *ifnar*^{-/-} Mice



TNX-801 infection did not produce any clinical disease



TNX-801 Infection of C57BL/6 *ifnar*^{-/-} Mice



TNX-801 infection did not produce any clinical disease

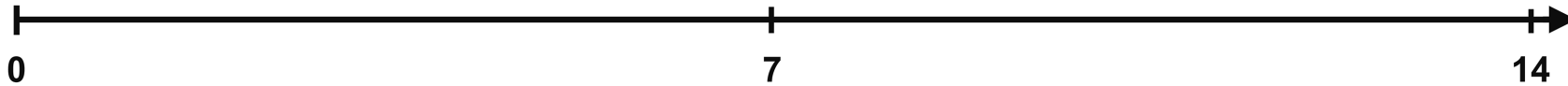


TNX-801 Infection of C57BL/6 *ifnar*^{-/-}/*ifngr*^{-/-} Mice

C57BL/6 *ifnar*^{-/-}/*ifngr*^{-/-}



Route: IN



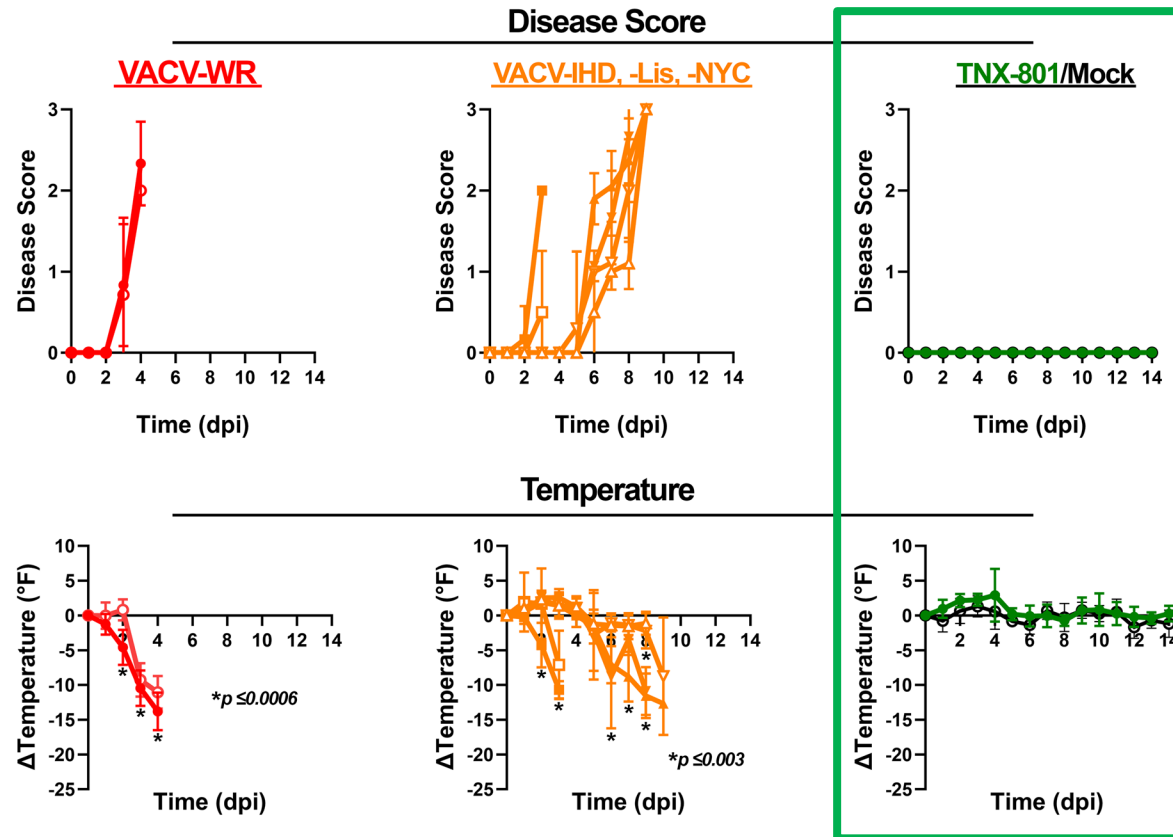
- VACV-WR (5.7 log₁₀ PFU)
- VACV-WR (4.8 log₁₀ PFU)
- VACV-IHD (6.6 log₁₀ PFU)
- VACV-IHD (5.7 log₁₀ PFU)
- VACV-Lis (5.9 log₁₀ PFU)
- VACV-Lis (5.0 log₁₀ PFU)
- VACV-NYCBH (5.8 log₁₀ PFU)
- ▲ VACV-NYCBH (5.0 log₁₀ PFU)
- TNX-801 (7.8 log₁₀ PFU)
- Mock

➤ **Parameters measured:**

- 1) Clinical Score
- 2) Temperature
- 3) Weight loss
- 4) Survival



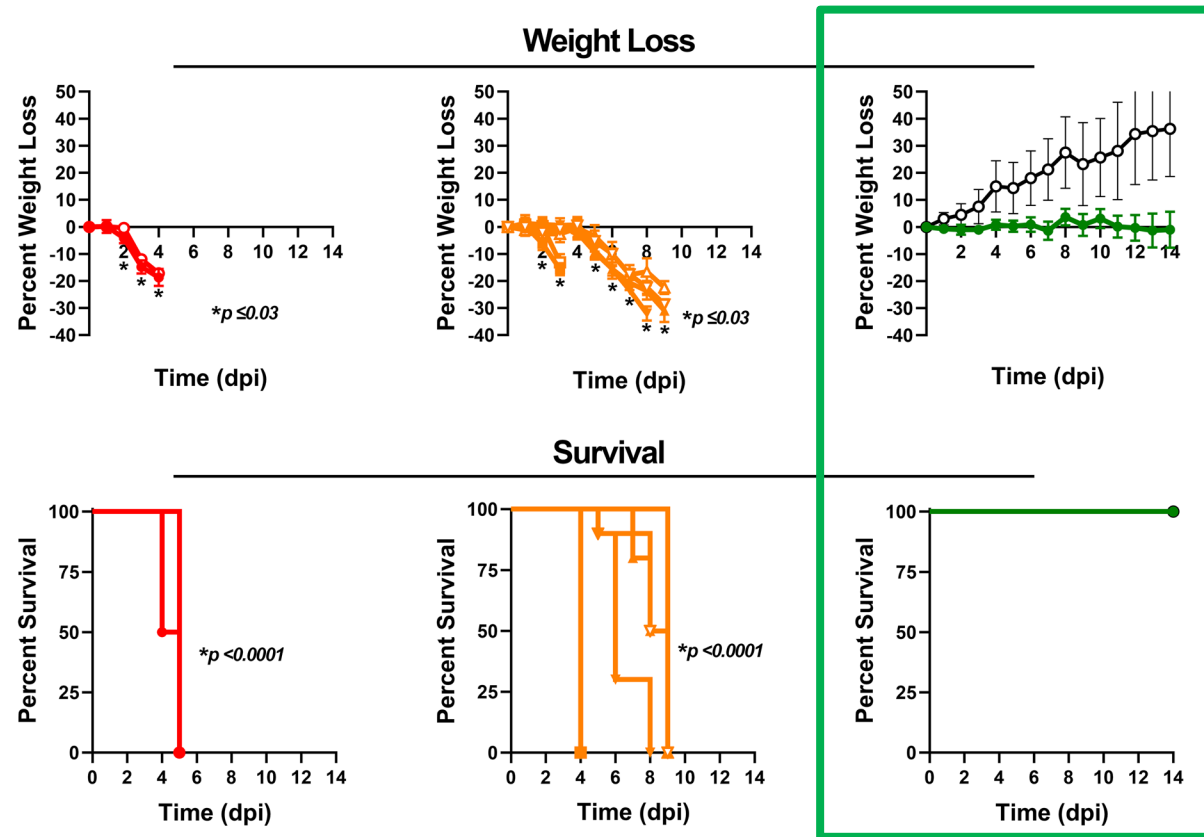
TNX-801 Infection of C57BL/6 *ifnar*^{-/-}/*ifngr*^{-/-} Mice



TNX-801 infection did not produce any clinical disease



TNX-801 Infection of C57BL/6 *ifnar*^{-/-}/*ifngr*^{-/-} Mice: >1,000-fold more Attenuated



TNX-801 infection did not produce any clinical disease



4) TNX-801 immunogenicity and efficacy in NHP model



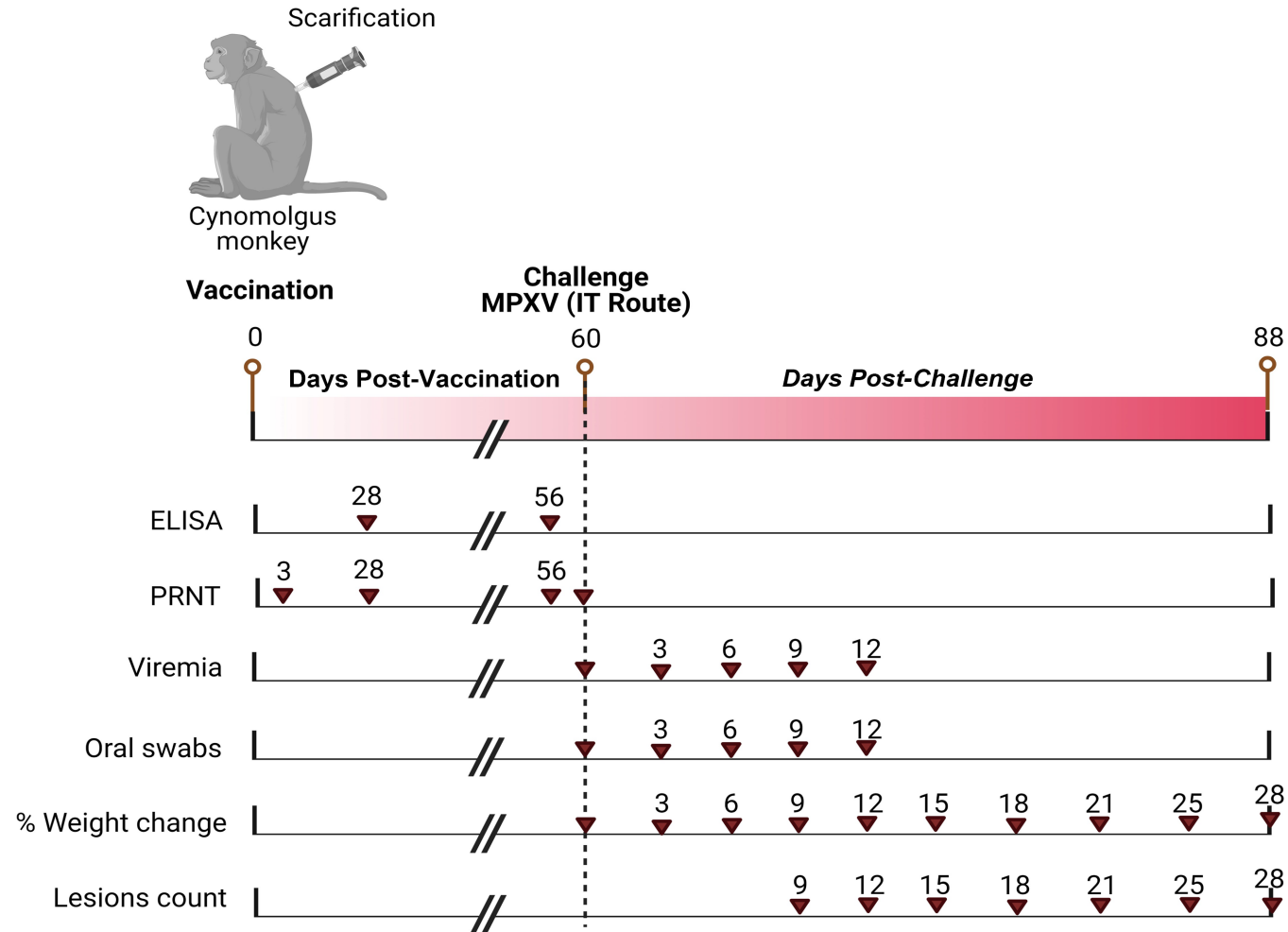
TNX-801 Immunogenicity and Efficacy in NHPs: Study Design

Vaccination					Challenge		
Group	Vaccine	N	Dose (Log ₁₀ PFU)	Route	Virus	Dose (Log ₁₀ PFU)	Route
1	TNX-801 (High)	4	6.6	Scarification	MPXV (Zaire)	5.0	IT
2	TNX-801 (Low)	4	5.7	Scarification	MPXV (Zaire)	5.0	IT
3	rVACV	4	5.0	Scarification	MPXV (Zaire)	5.0	IT
4	Mock	4	-	Scarification	MPXV (Zaire)	5.0	IT

rVACV = Plaque pick from ACAM2000 (Approved Vaccine)

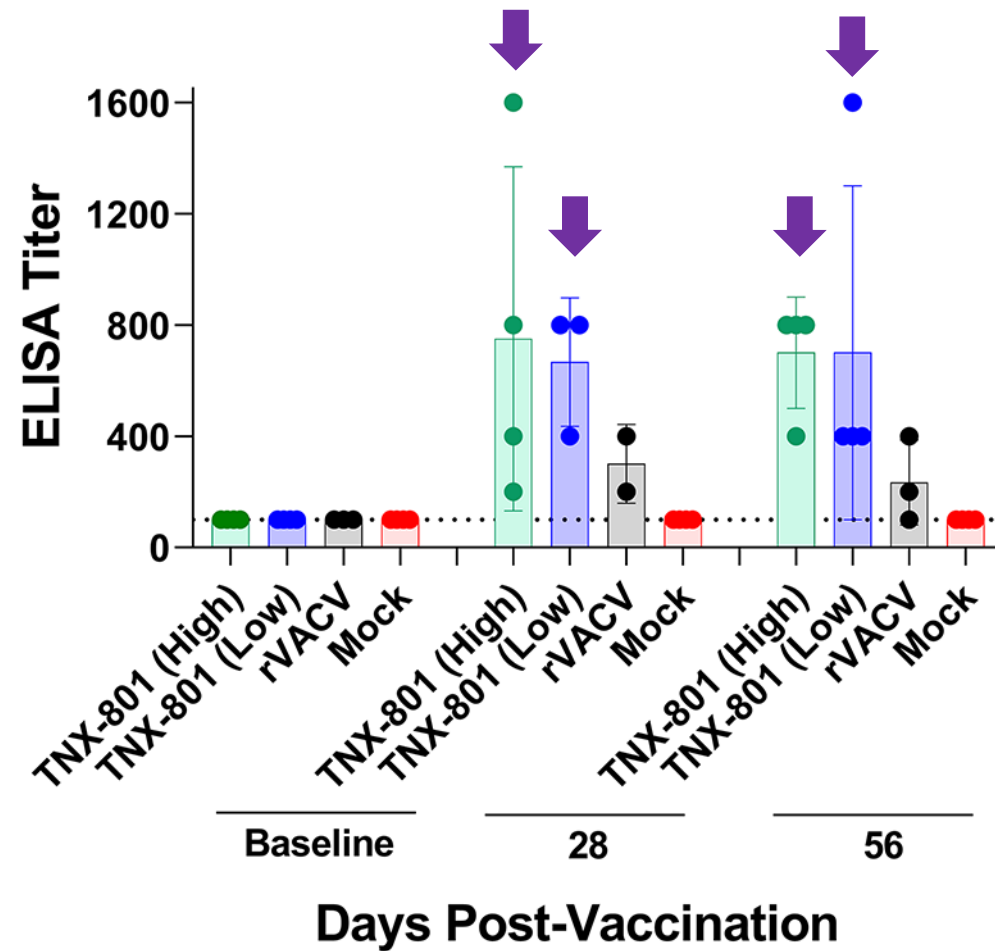


Immunogenicity and Efficacy in NHPs – Study Design





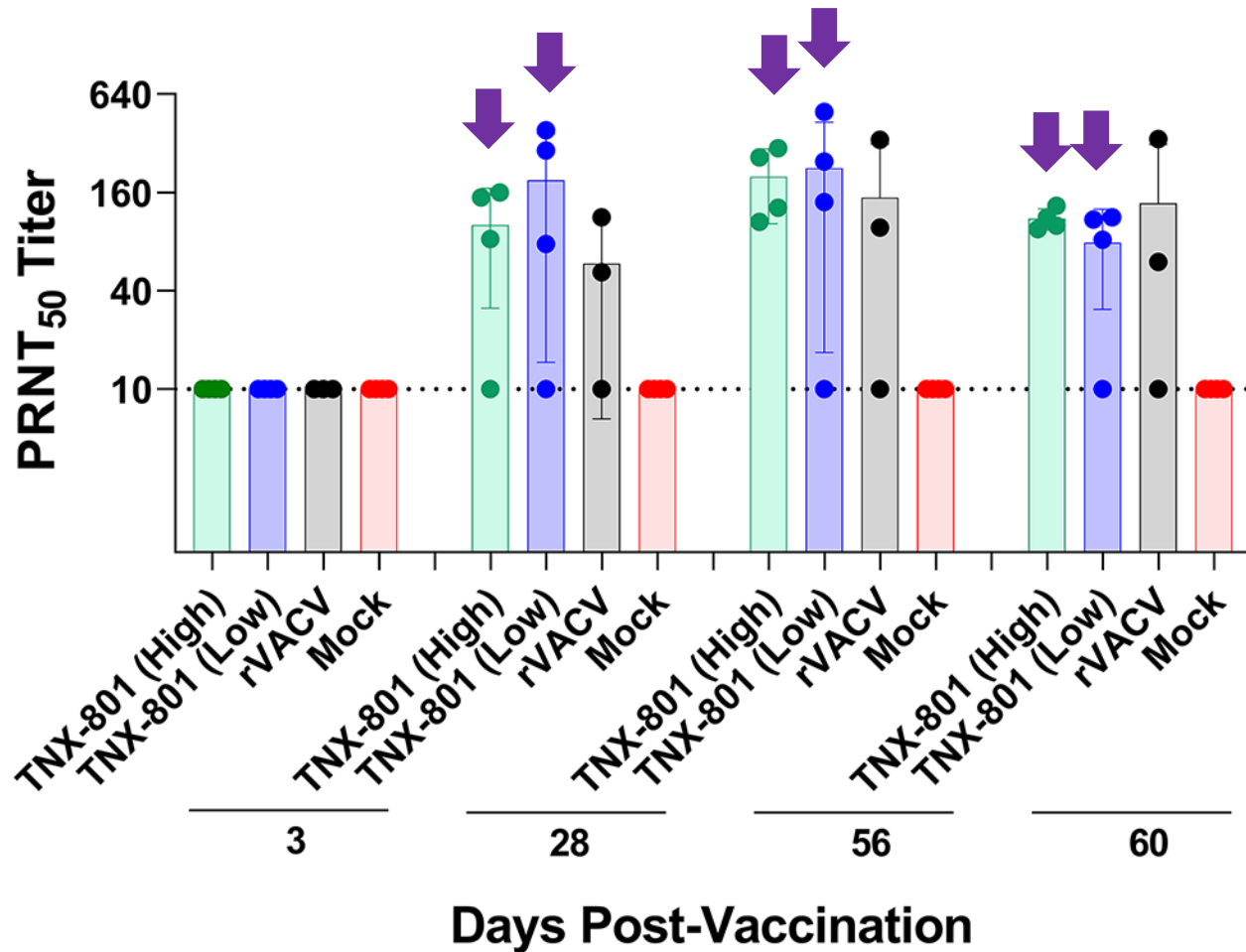
Immunogenicity: Total IgG (ELISA)



↓ **≡ 100% Seroconversion**
in TNX-801 vaccinated
groups



Immunogenicity: Neutralizing Antibody (PRNT₅₀ Assay)

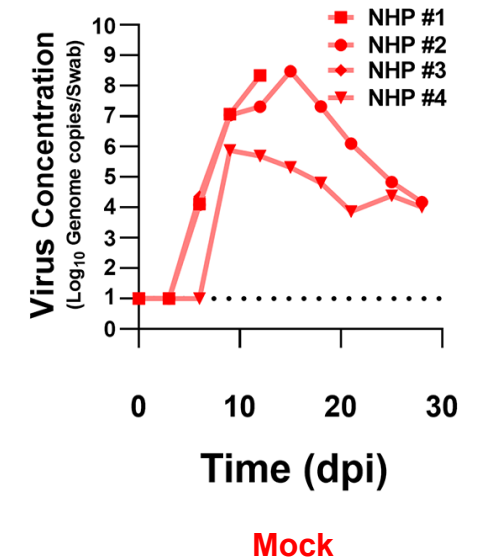
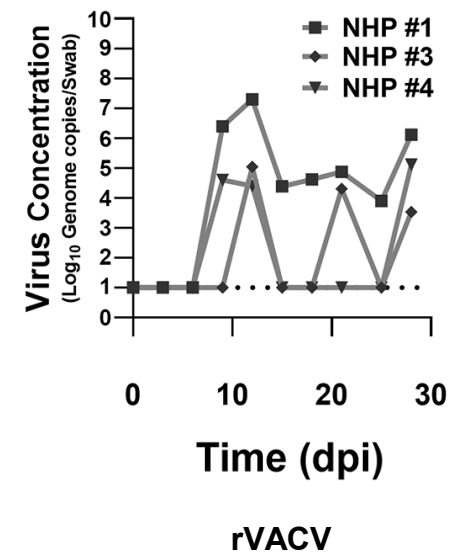
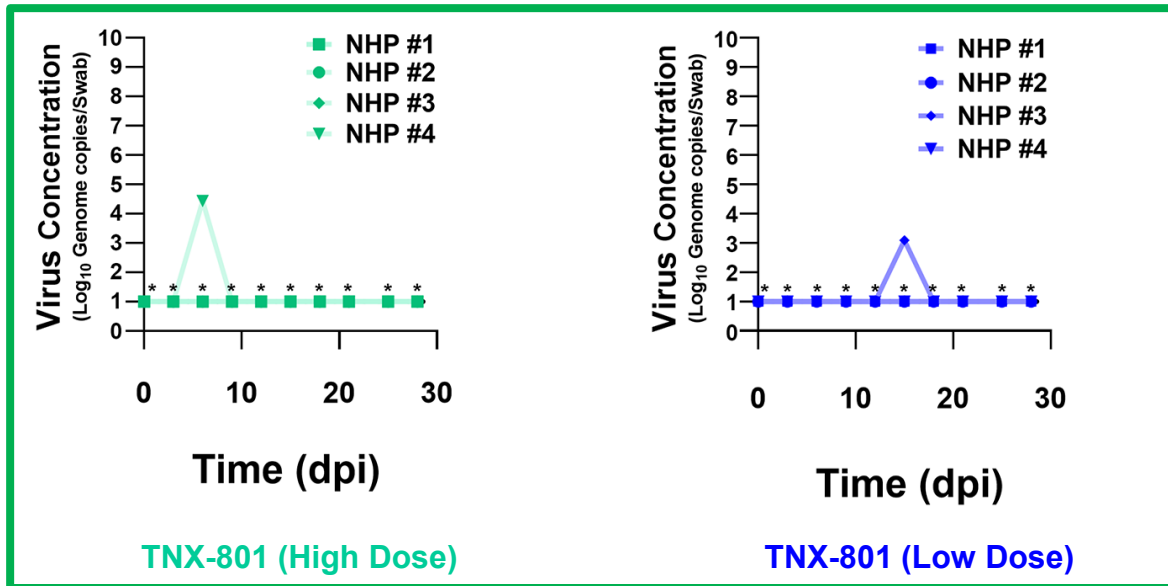


↓ **≡ 88% NHPs in TNX-801 vaccinated groups had neutralizing antibody responses**



Virus Shedding: Oral Swabs (qPCR)

Oral Swabs

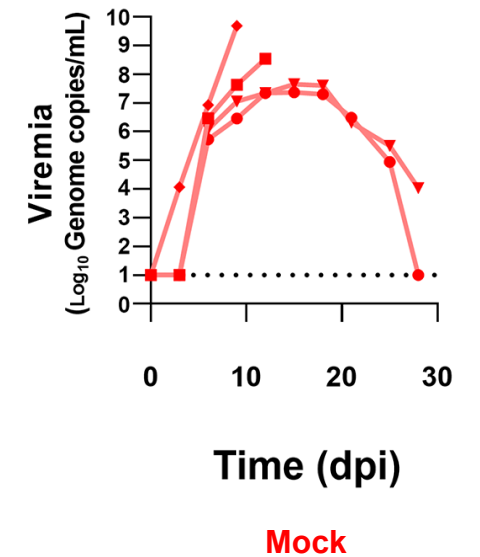
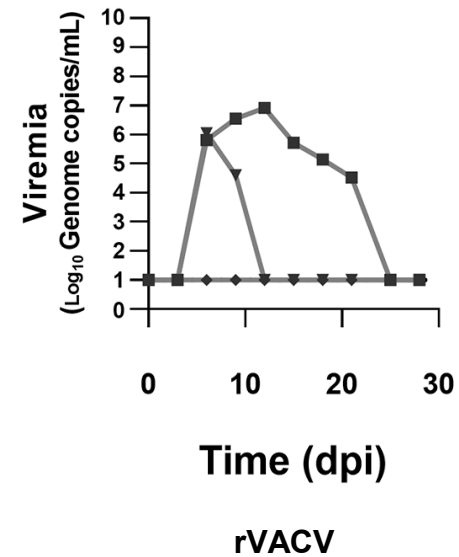
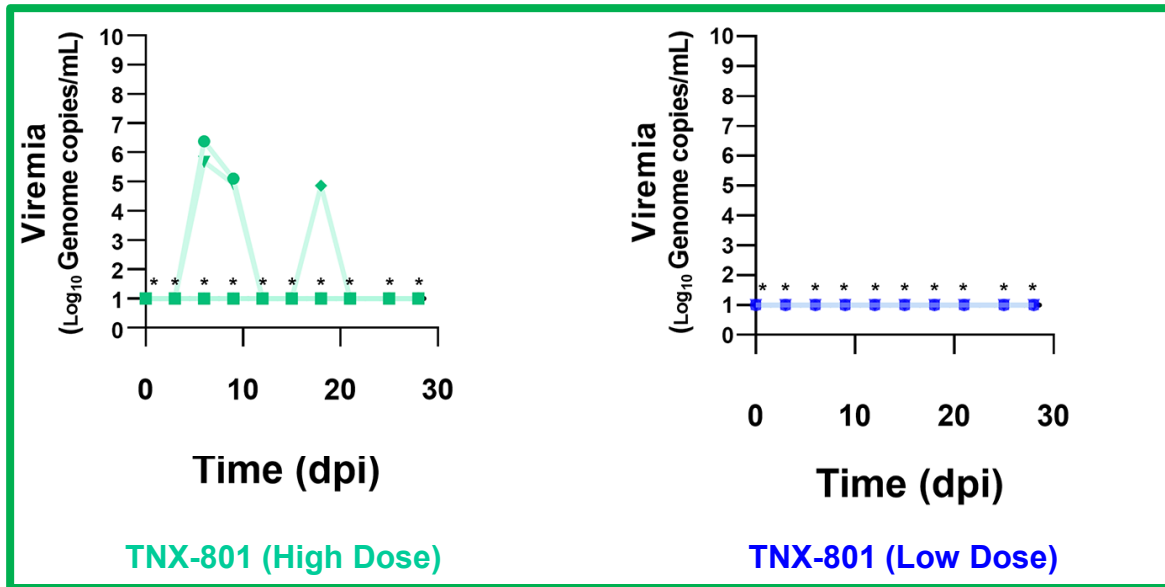


Minimal or no virus shedding in TNX-801 vaccinated groups



Viremia (qPCR)

Viremia

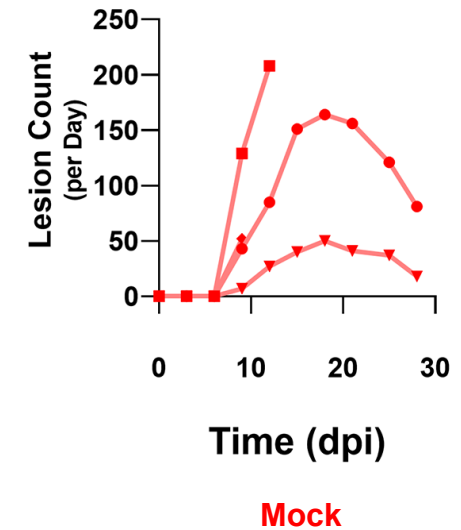
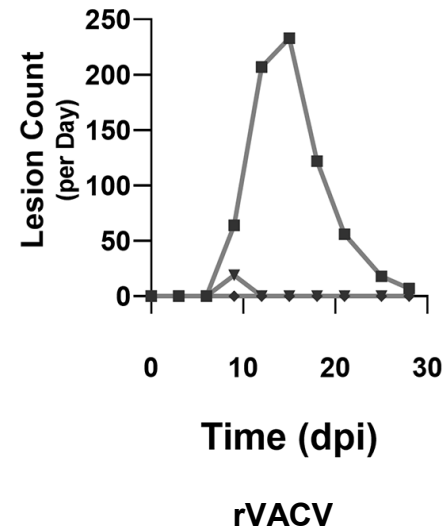
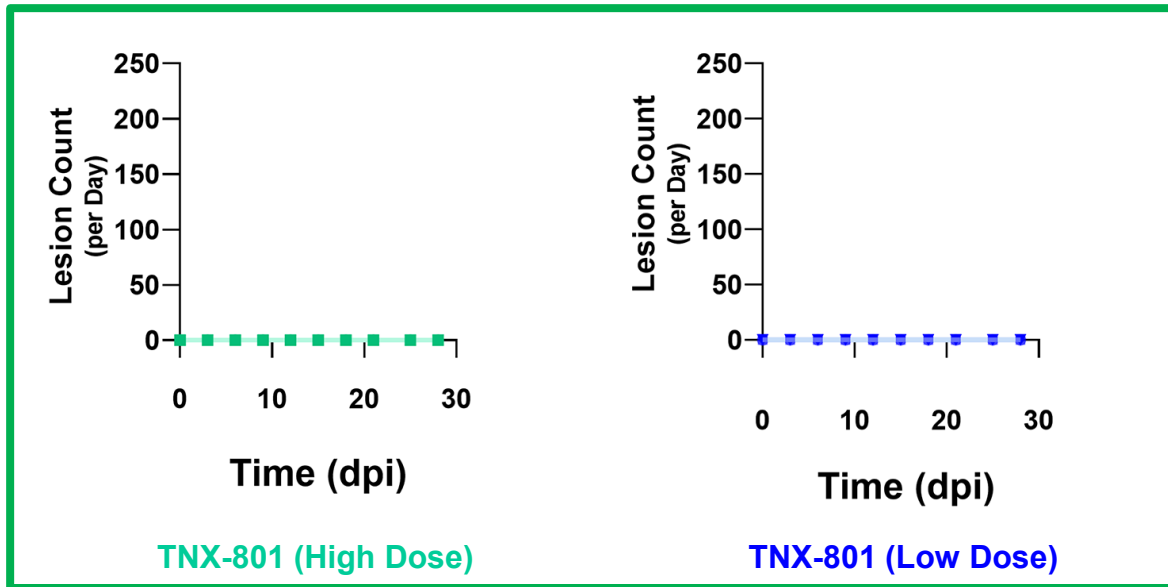


Sporadic or no viremia in TNX-801 vaccinated groups



Clinical Disease: Lesions

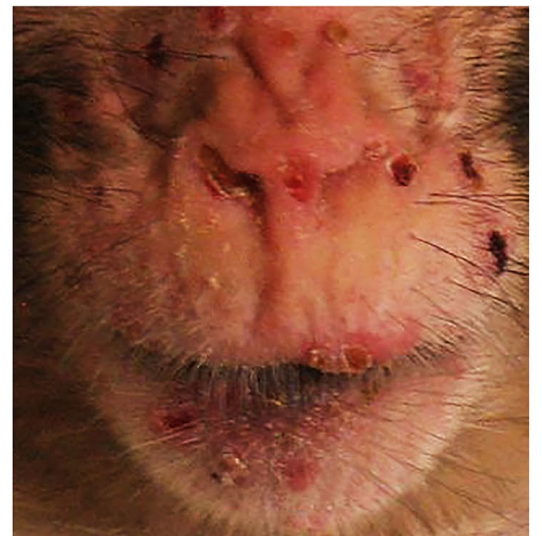
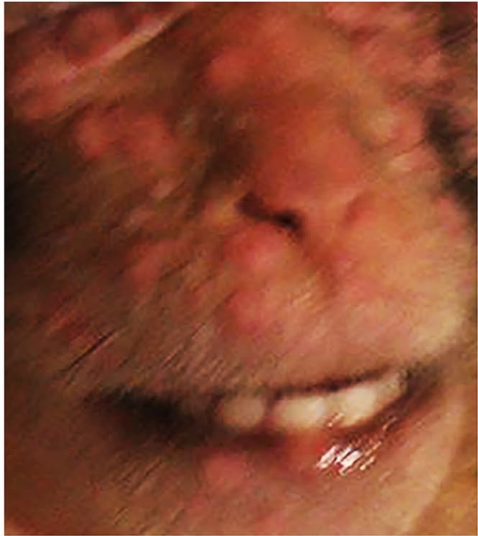
Lesions



NO LESIONS in TNX-801 vaccinated groups

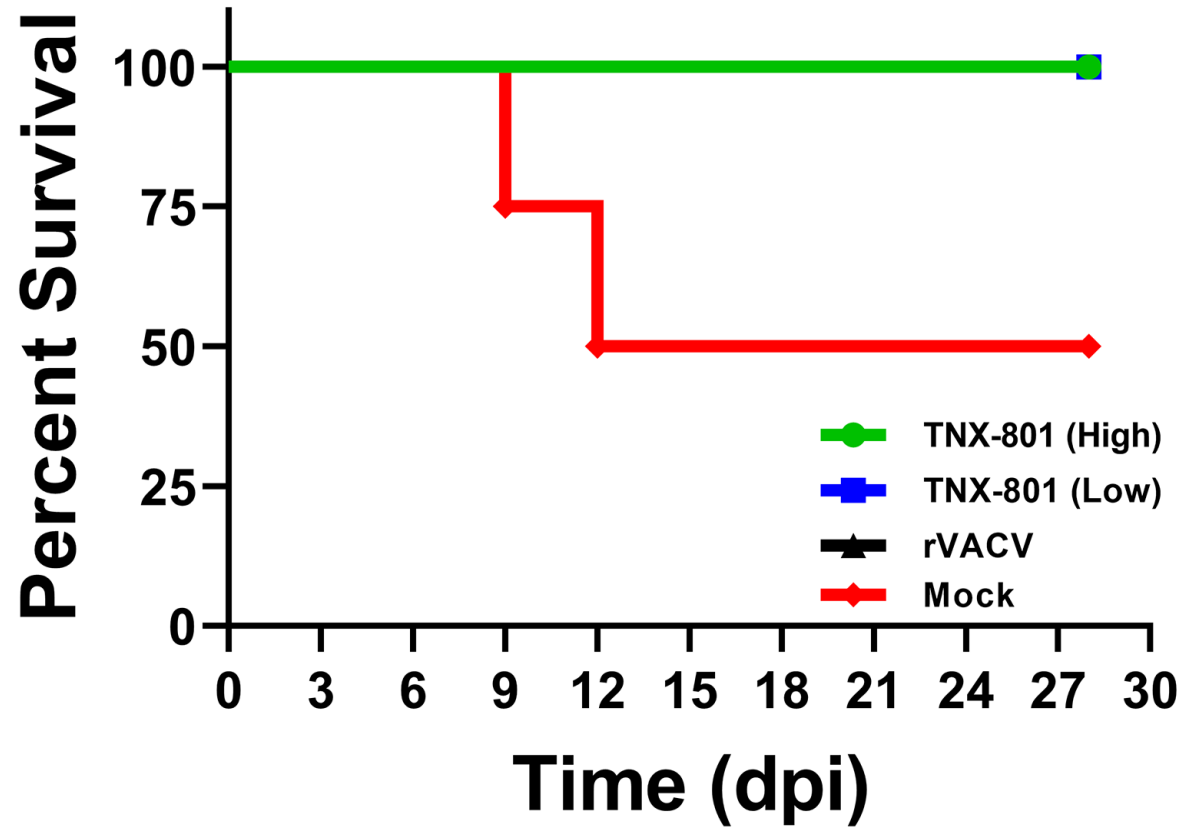


Clinical Disease: Lesions (Mock Group)





Clinical Disease: Lethality



NO LETHALITY in TNX-801 vaccinated groups



Conclusions

➤ **TNX-801 is attenuated *in vitro*:**

- 1) Plaque phenotype: VACV (~3-4 mm) vs. TNX-801 (~1-2 mm)
- 2) Multi-step growth kinetics:
 - Immortalized cell lines: ~10- to 100-fold attenuation
 - Human primary cell lines: ~5- to 100-fold attenuation

➤ **TNX-801 is attenuated *in vivo*:**

- 1) Assessed TNX-801 attenuation in immunocompromised murine models (C57BL/6 *ifnar*^{-/-} and C57BL/6 *ifnar*^{-/-}/*ifngr*^{-/-})
- 2) TNX-801 is >100- to 1,000-fold more attenuated than VACV strains
- 3) TNX-801 is indistinguishable from mock treated animals in immunocompromised model



Conclusions (NHP Study)

- A single dose of TNX-801 vaccination was well tolerated
 - No severe adverse events (no lesions) following vaccination
- TNX-801 vaccination via traditional route (scarification) was immunogenic
- Provided near complete protection against virus shedding, viremia, and weight loss
- No clinical disease was observed (lesions)
- All NHPs (TNX-801 and rVACV) survived lethal challenge



Acknowledgements

➤ Tonix Pharmaceuticals

- Stephanie Trefry
- Mayanka Awasthi
- Christy Raney
- Amy Cregger
- Chase A Gonzales
- Brittney Layton
- Robert Enamorado
- Nelson Martinez
- Deborah Gohegan
- Masoudeh Masoud-Bahnamiri

➤ Tonix Pharmaceuticals

- Jennifer Cho
- Dawn Myscofski
- Tinoush Moulaei
- Natasza Ziólkowska
- Scott Goebel
- Siobhan Fogarty
- Helen Stillwell
- Bruce Daugherty
- Seth Lederman

➤ University of Alberta

- Ryan Noyce
- David Evans

➤ Southern Research