TONIX PHARMACEUTICALS

The Development of Horsepox Virus as a Vaccine Platform: Evaluation of TNX-1800 as a SARS-CoV-2 Vaccine

Farooq Nasar, Ph.D., M.P.H.
Senior Principal Scientist

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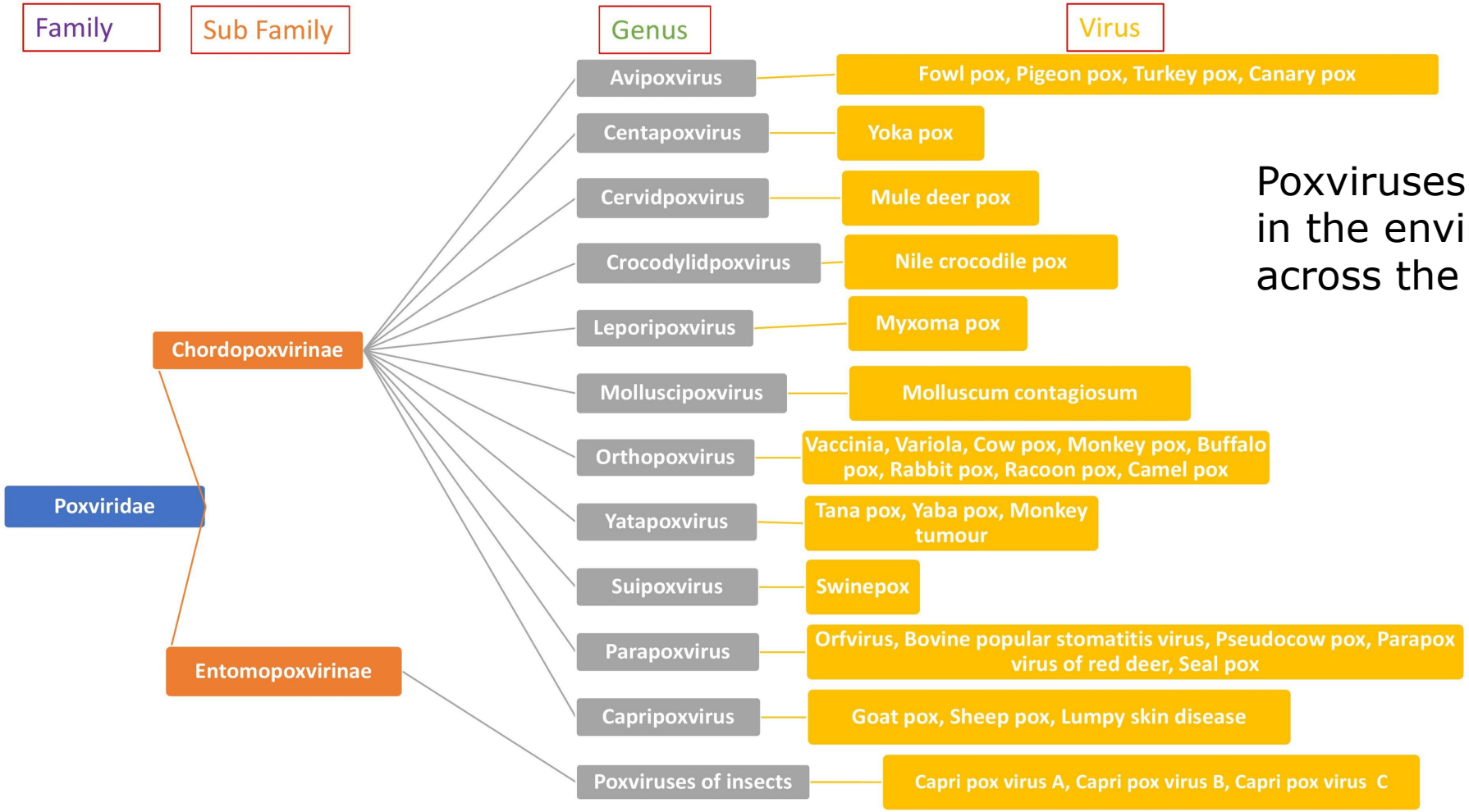
Poxviruses

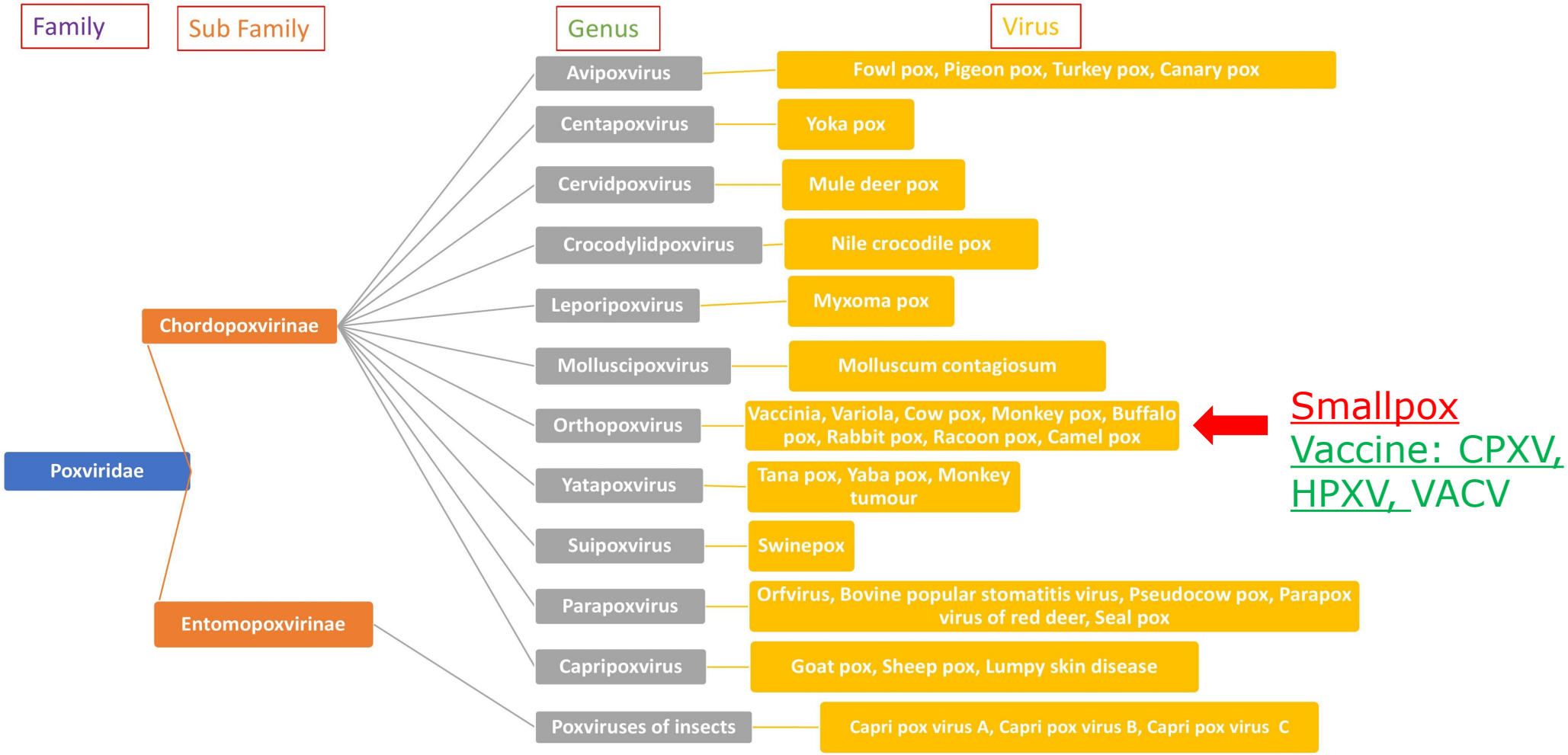
- Family: ***Poxviridae***
- Two subfamilies:
    1) *Chordopoxvirinae*
    2) *Entomopoxvirinae*
- 22 Genera
- Double stranded DNA, enveloped, ~128-380kb
- Virions: brick-shaped, ~250 x 350 nm
- Infect vertebrate and invertebrate hosts

Poxviruses



Poxviruses are ubiquitous in the environment across the globe

Orthopox Viruses

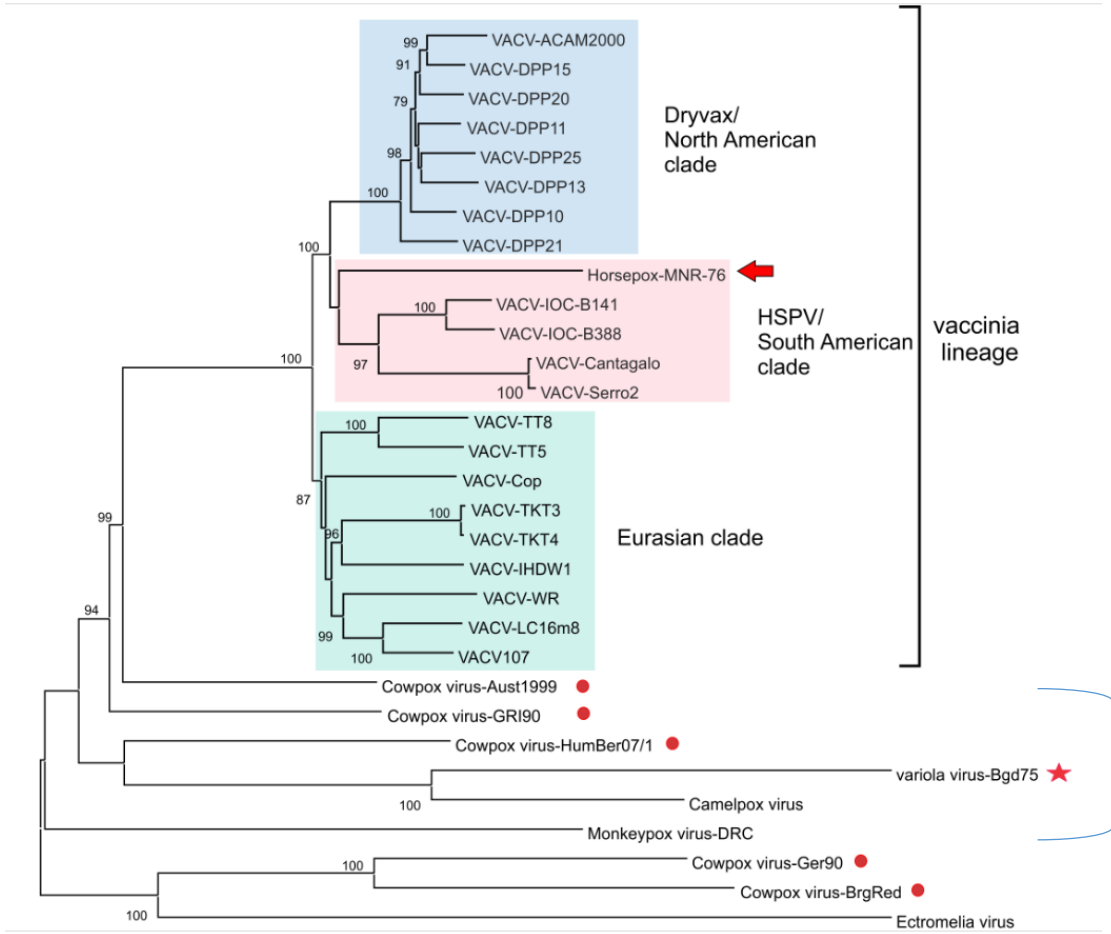


In 1796, Edward Jenner Successfully Used Vaccination to Protect Against Smallpox

- 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**, actually originated in horses and had been transferred from horses to cows' udders by dirty hands

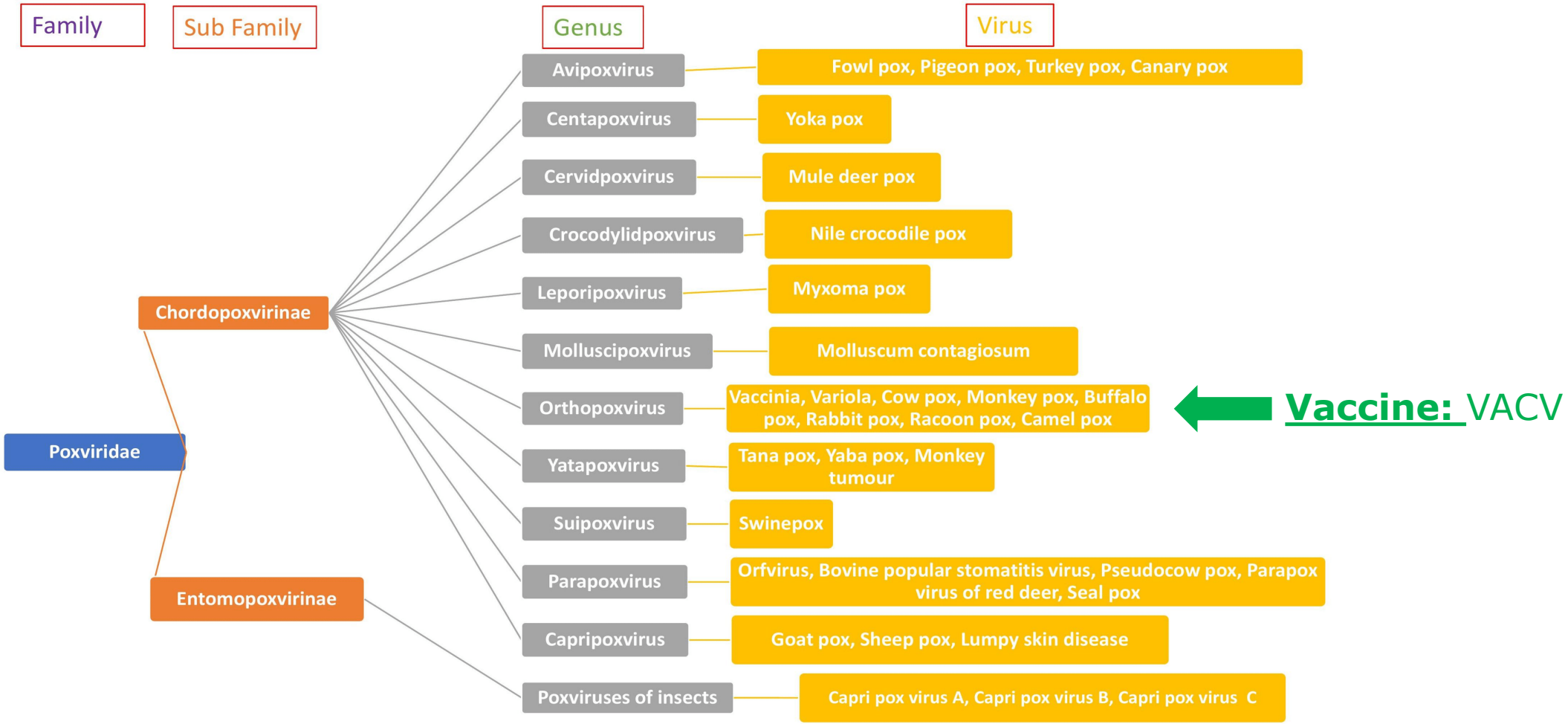
The College of Physicians of Philadelphia. Accessed July 15, 2021. https://www.historyofvaccines.org

Phylogenetic Tree of Genus *Orthopox*



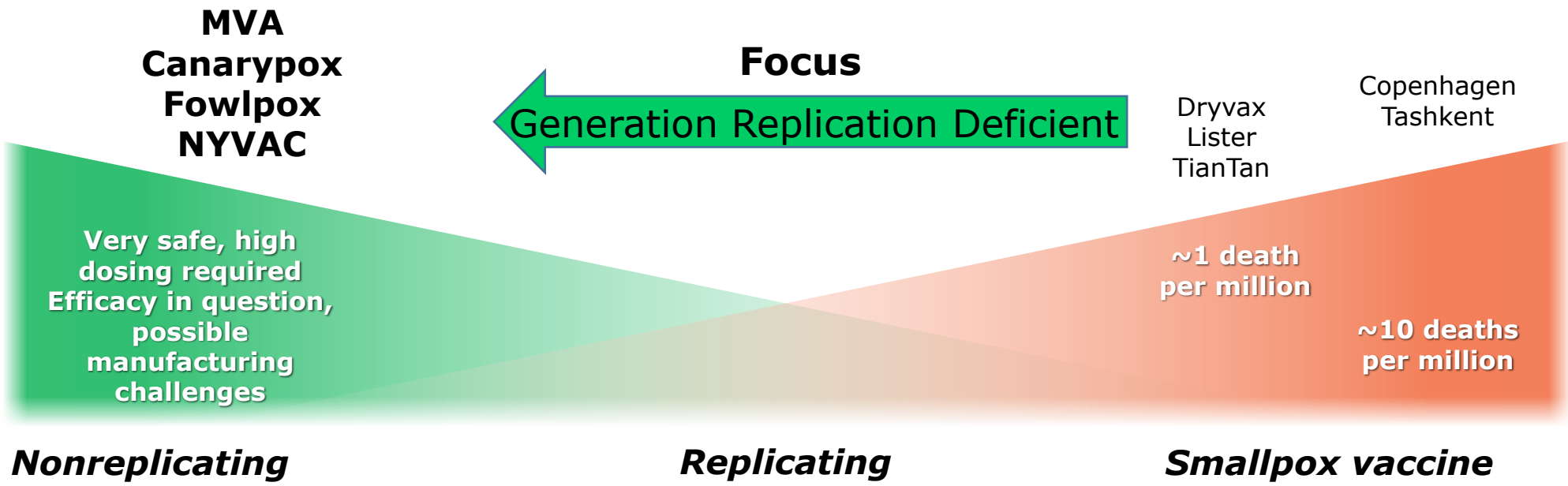
Cowpox close orthopox relative to smallpox (Variola)
Conferred protection against Variola

Orthopox Viruses; Poxvirus-based Vaccines



Recombinant Pox-based Vector Development Addressing "Safety" minimization of Adverse Events

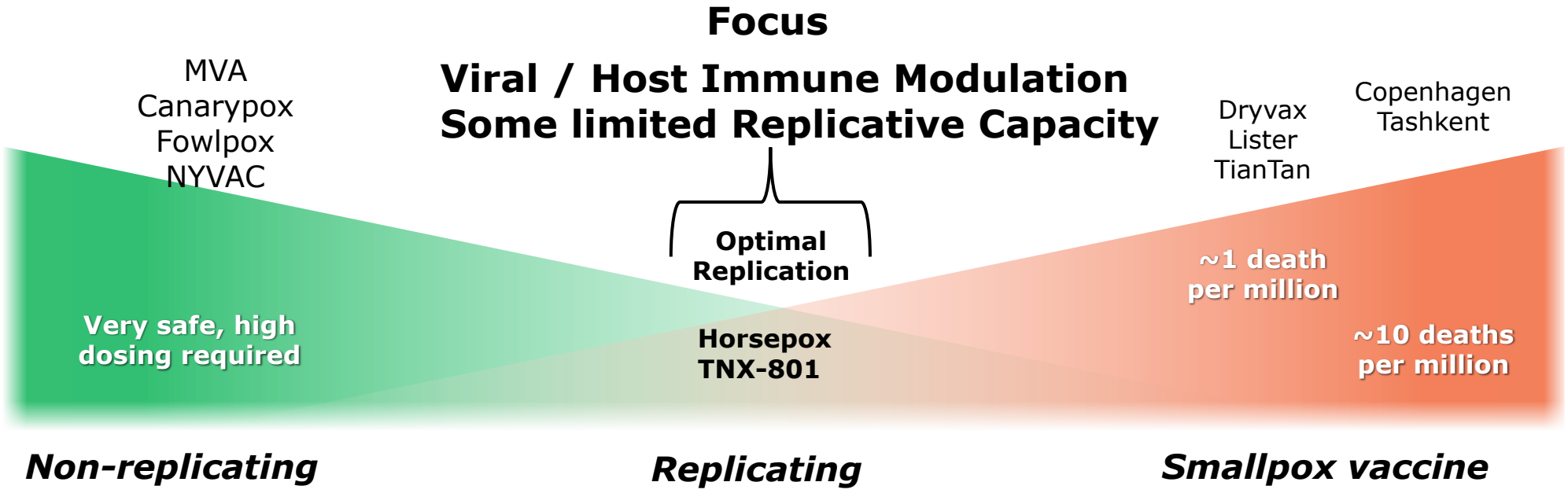
- Three decades pox-vector modifications and engineering focused on the generation of *replication deficient (RD) vectors*



Recombinant Pox-based Vector Development Addressing "Safety" minimization of Adverse Events

Considering the overall body of data from RD pox-based vectors
Have we gone to far in vector engineering requiring *RD*?

- Safety data is great but immunological responses are typically weak or suboptimal immune responses
- *Some Replicative Capacity is essential, Horsepox TNX-801**



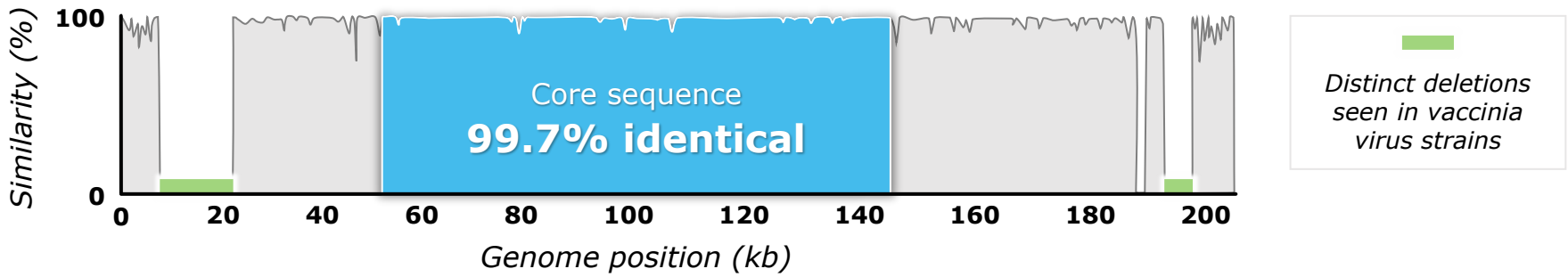
*TNX-801 has not been approved for any indication.

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 Europe[1]
- Vaccine producers may have propagated stocks by periodically supplementing or refreshing them with horsepox[2]

A 1902 smallpox vaccine (**Mulford**) was found to be **99.7% identical to HPVX** in core viral sequence, implicating a HPXV-like virus as a progenitor to modern vaccinia[3]

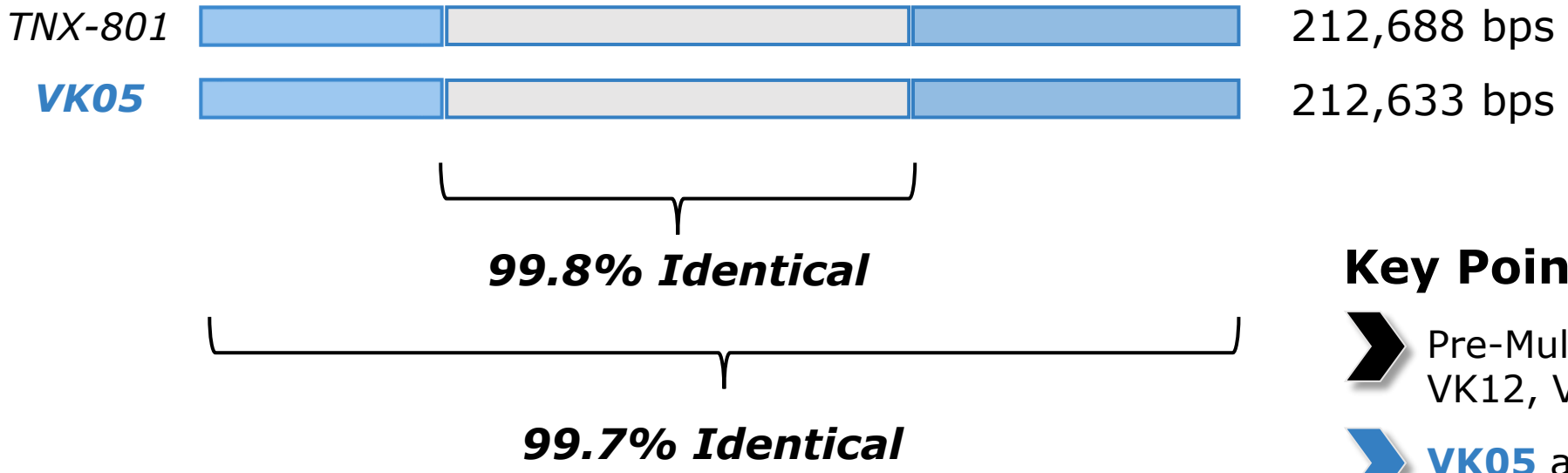
Sequence Identity for the 1902 Mulford Vaccine Compared to HPVX[3]



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 and HPXV-Like Viruses Were Used as Civil War-Era (1860s-1870s) Vaccines

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



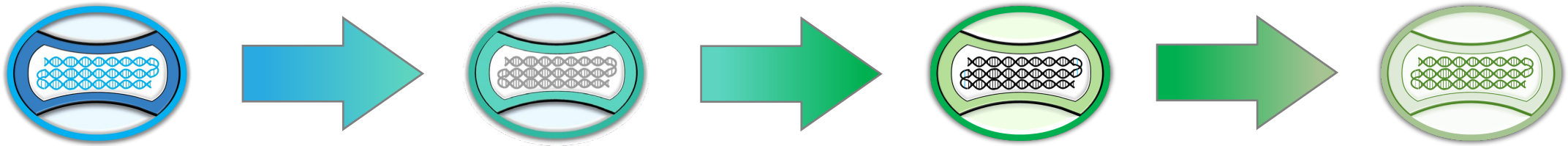
Key Points

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

Brinkmann A, et al. *Genome Biol*. 2020;21(1):286.

Evolution of the Vaccinia Genome

- Recent studies demonstrate that HPXV and HPXV-like viruses were used as smallpox vaccines in the 1800s[1,2]



HPXV or HPXV-like

Jenner's vaccinia (agent of "cowpox")

1902 Mulford vaccine

Modern vaccinia

Civil War-era vaccines are genetically diverse, with some strains having 1 or both ITRs intact

One recently identified strain, VK05, was found to be **99.7% idetical to HPXV** across the whole genome

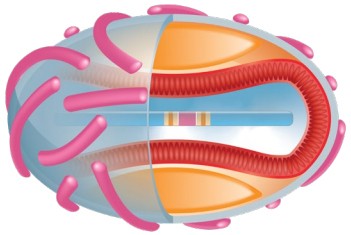
The 1902 Mulford vaccine is similar to both horsepox (99.7% identical core sequence) and vaccinia (**similar ITR deletions**)

Selected for WHO Eradication Intensification Program (1967)

1. Duggan AT, et al. *Genome Biol.* 2020;21(1):175.
2. Brinkmann A, et al. *Genome Biol.* 2020;21(1):286.

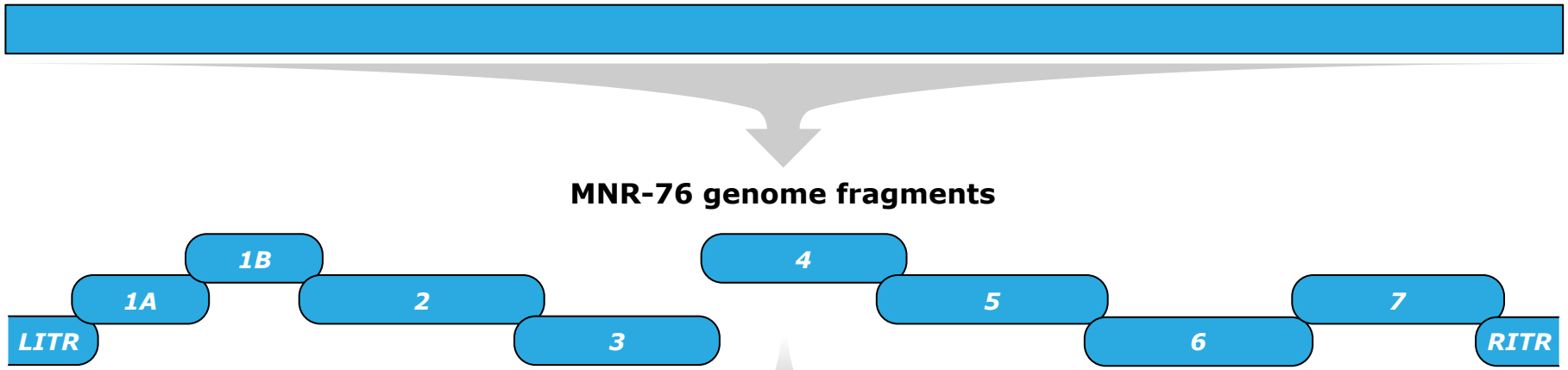
TNX-801 Core Genome Is Identical to the Published HPXV Strain MNR-76

The core genome of TNX-801 is identical to MNR-76[1]



TNX-801
scHPXV (Horsepox)
212,811 bp

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



Ten overlapping genome fragments were assembled based on sequence homology to generate the TNX-801 genome[1,2]

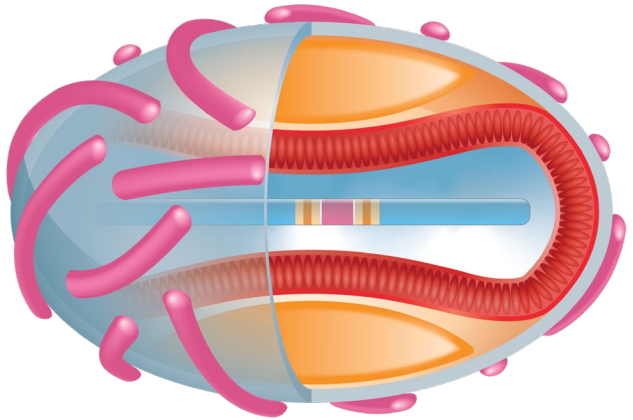
TNX-801 genome (212,811 bp) *Genbank accession KY349117*

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.

Properties of TNX-801 Live HPXV Vaccine

- TNX-801 is a vaccine based on sequence of isolated HPXV clone MNR-76[1,2]
  - The core genome of TNX-801 is identical to MNR-76, with ~70 bp terminal hairpin sequences from vaccinia added due to incomplete sequencing of MNR-76[1,2]
  - Small plaque size in culture (suggesting lower virulence) that appears similar to the CDC publication of the 1976 horsepox isolate MNR-76[3]
  - Substantially decreased virulence in mice relative to a vaccinia-based vaccine strain[2]
  - Protects macaques from monkeypox with no overt sign of clinical symptoms and no lesions in 8/8 animals at 2 doses of TNX-801[4]

TNX-801



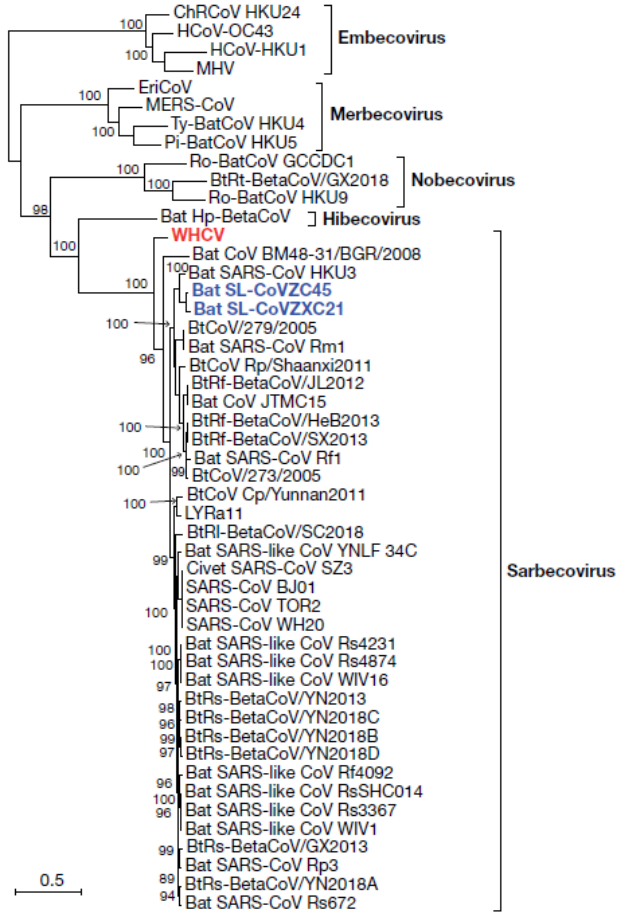
Horsepox Virus

scHPXV *(212 kb)*

1. Tulman ER, et al. *J Virol.* 2006;80(18):9244-58.
2. Noyce RS, et al. *PLoS One.* 2018;13(1):e0188453.
3. Trindade GS, et al. *Viruses.* 2016;8(12):328.
4. Noyce, RS, et al. Poster presented at: American Society of Microbiology BioThreats Conference; January 29, 2020; Arlington, VA. 114.

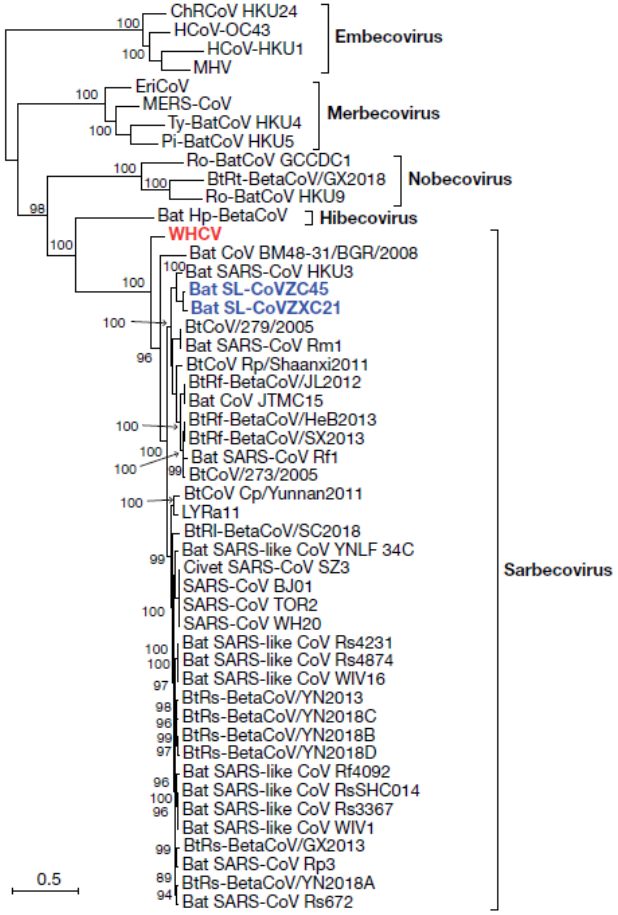
SARS-CoV-2

- SARS-CoV-2 emerged from Wuhan, China in 2019/2020
- Family: *Coronaviridae*
  - Genus: *Betacoronavirus*
  - Positive sense, single stranded, RNA virus
  - Genome: ~30kb



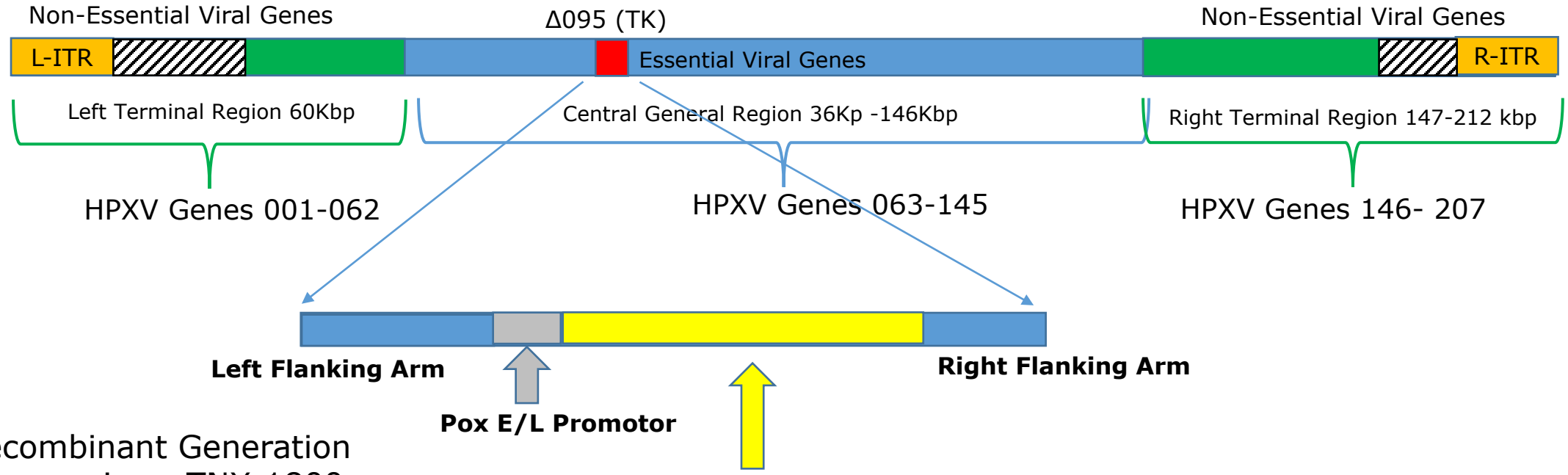
SARS-CoV-2

- SARS-CoV-2 emerged from Wuhan, China in 2019/2020
- Family: *Coronaviridae*
  - Genus: *Betacoronavirus*
  - Positive sense, single stranded, RNA virus
  - Genome: ~30kb
- ***Develop HPXV vaccine platform***
  - Model system: SARS CoV-2
  - "Proof of concept"
  - Encoding Spike protein (WA-2020)
  - ***TNX-1800***



Recombinant SARS-CoV-2 Vaccine Generation (TNX-1800*)

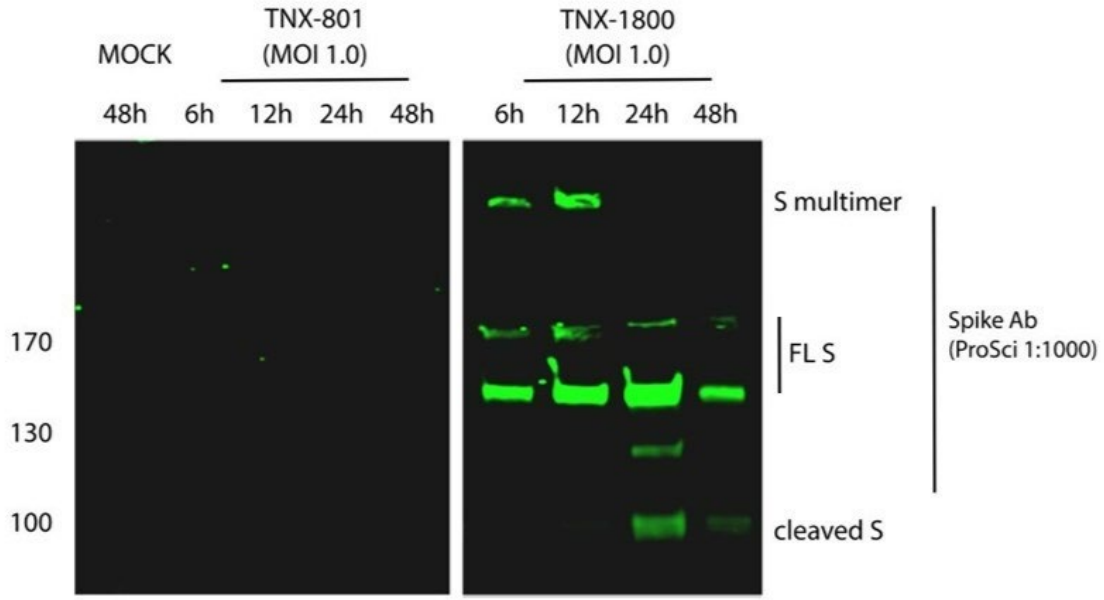
Development of HPXV as a recombinant Delivery Vector Platform



Recombinant Generation
Rescue virus: TNX-1800

*TNX-1800 has not been approved for any indication.

Recombinant Vaccine Expressing Heterologous Antigen (TNX-1800): Spike Protein Expression



TNX-1800 rapidly expresses SARS-CoV-2 spike protein

Preliminary Immunogenicity Studies

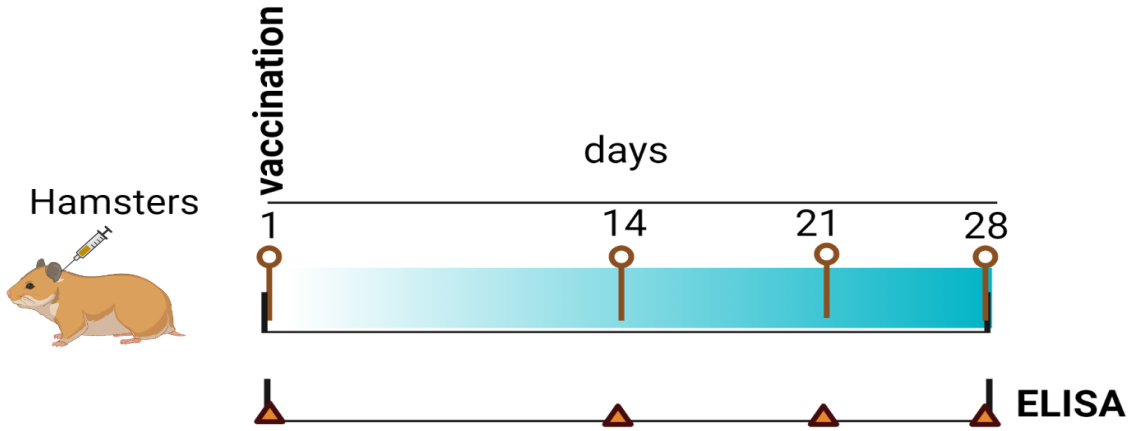
- Goal: *Investigate immunogenicity and tolerability following administration of a single dose of TNX-1800*

- Two animal models:

1) Syrian Hamsters

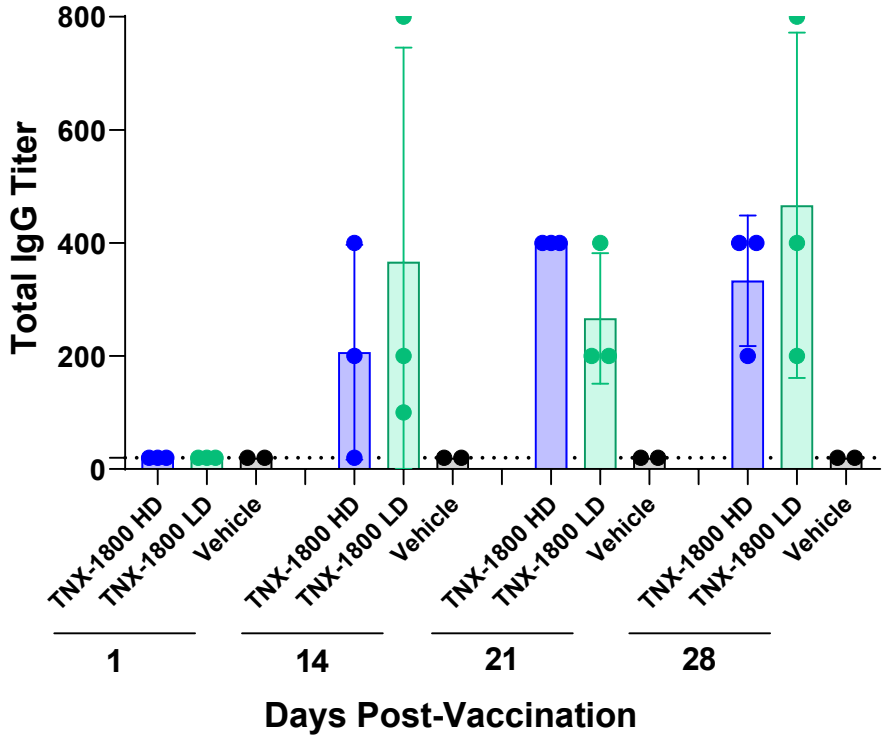
2) New Zealand Rabbits

Preliminary Immunogenicity: Hamster Study Design



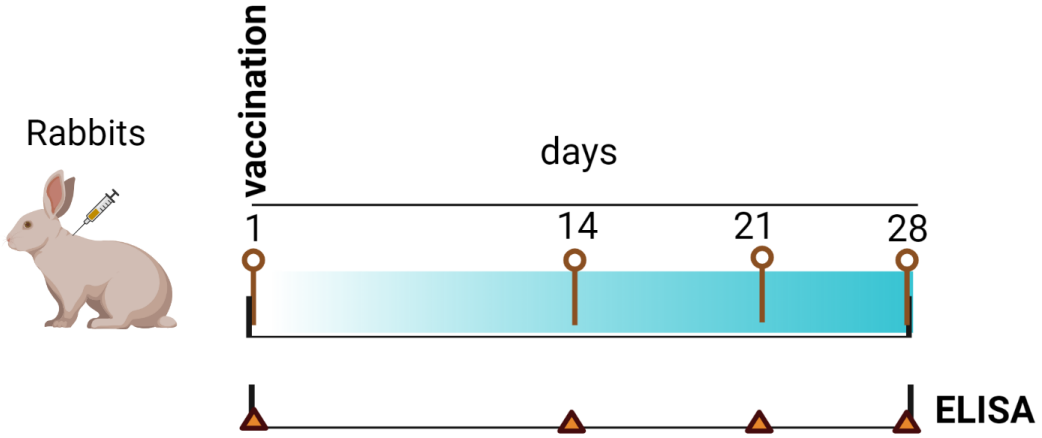
Vaccination in Hamsters				
Group	Vaccine	Number	Dose (log_{10} PFU/animal)	Route
1	TNX-1800 (HD)	2M/1F	6.5	Percutaneous
2	TNX-1800 (LD)	2M/1F	5.5	Percutaneous
3	Vehicle	1M/1F	-	Percutaneous

Preliminary Immunogenicity: SARS CoV-2 Spike Protein Specific ELISA Titers



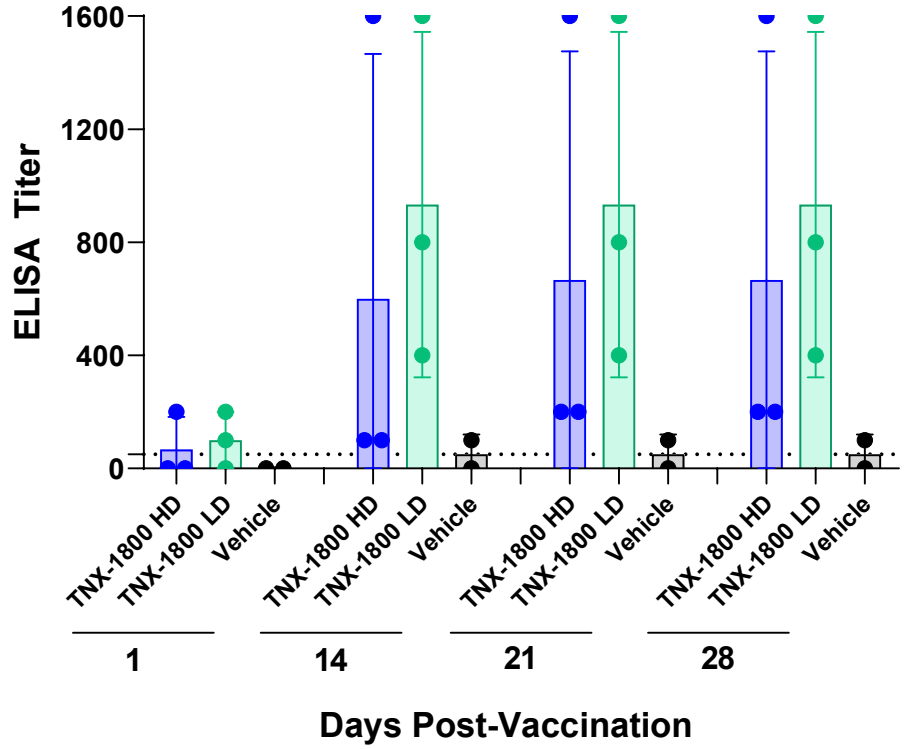
100% Hamsters in TNX-1800 vaccinated group had IgG antibody response

Preliminary Immunogenicity: Rabbit Study Design



Vaccination in Rabbits				
Group	Vaccine	Number	Dose (log_{10} PFU/animal)	Route
1	TNX-1800 (HD)	2M/1F	6.5	Percutaneous
2	TNX-1800 (LD)	2M/1F	5.5	Percutaneous
3	Vehicle	1M/1F	-	Percutaneous

Preliminary Immunogenicity: SARS CoV-2 Spike Protein Specific ELISA Titers



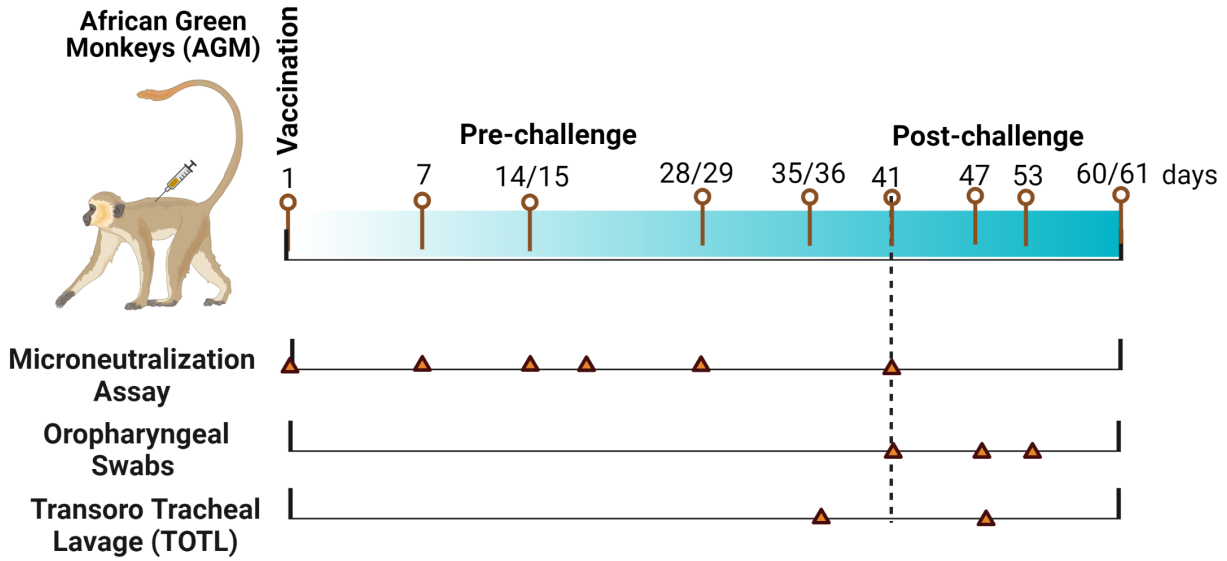
100% Rabbits in TNX-1800 vaccinated group had IgG antibody response

Preliminary Immunogenicity Studies: Conclusion

1) 100% of animals generate an antibody response
2) Vaccine was well-tolerated
   - No adverse events
   - No disseminated horsepox virus infection

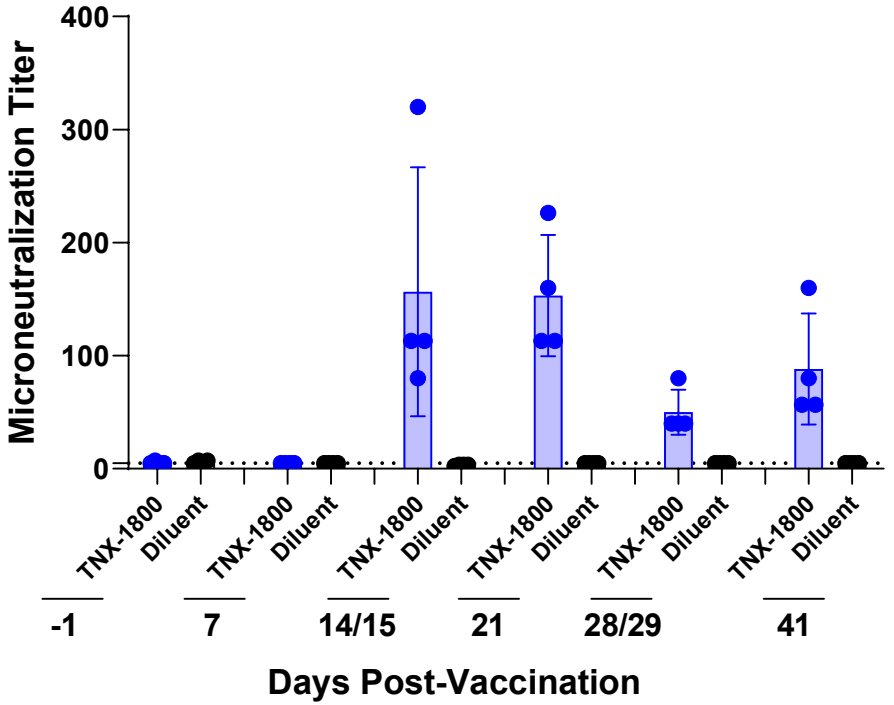
➢ *Proceeded to efficacy studies in NHPs*

Preliminary Efficacy Study Design: African Green Macaques



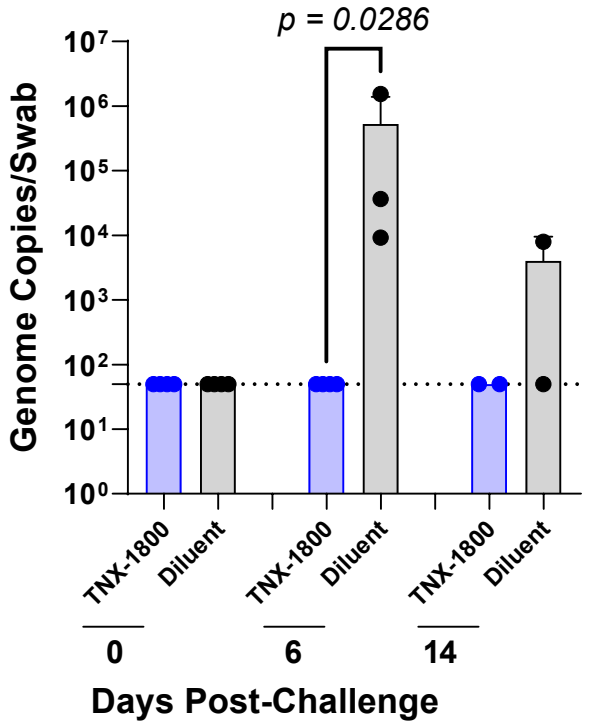
Vaccination					Challenge		
Group	Vaccine	N	Dose (Log_{10} PFU)	Route	*SARS-CoV-2 Challenge strain*	*Dose (Log_{10} PFU)*	*Route*
1	Diluent	4	Sham	Percutaneous	*USA-WA1/2020*	*6.3*	*IT/IN*
2	TNX-1800	4	6.5	Percutaneous	*USA-WA1/2020*	*6.3*	*IT/IN*

Immunogenicity: Neutralization Titers



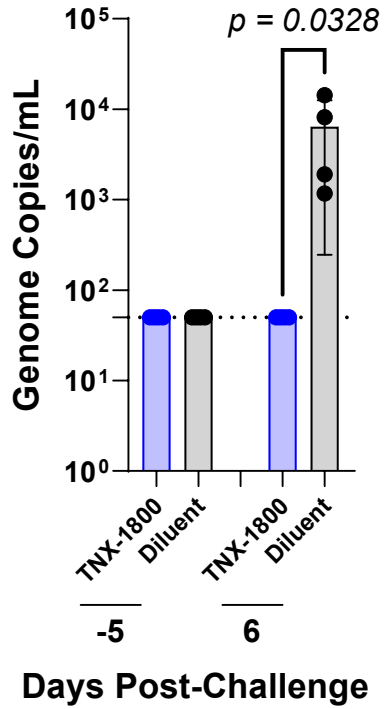
100% NHPs in TNX-1800 vaccinated group had neutralizing antibody response

Virus Replication/Shedding: Oropharyngeal (OP) swabs



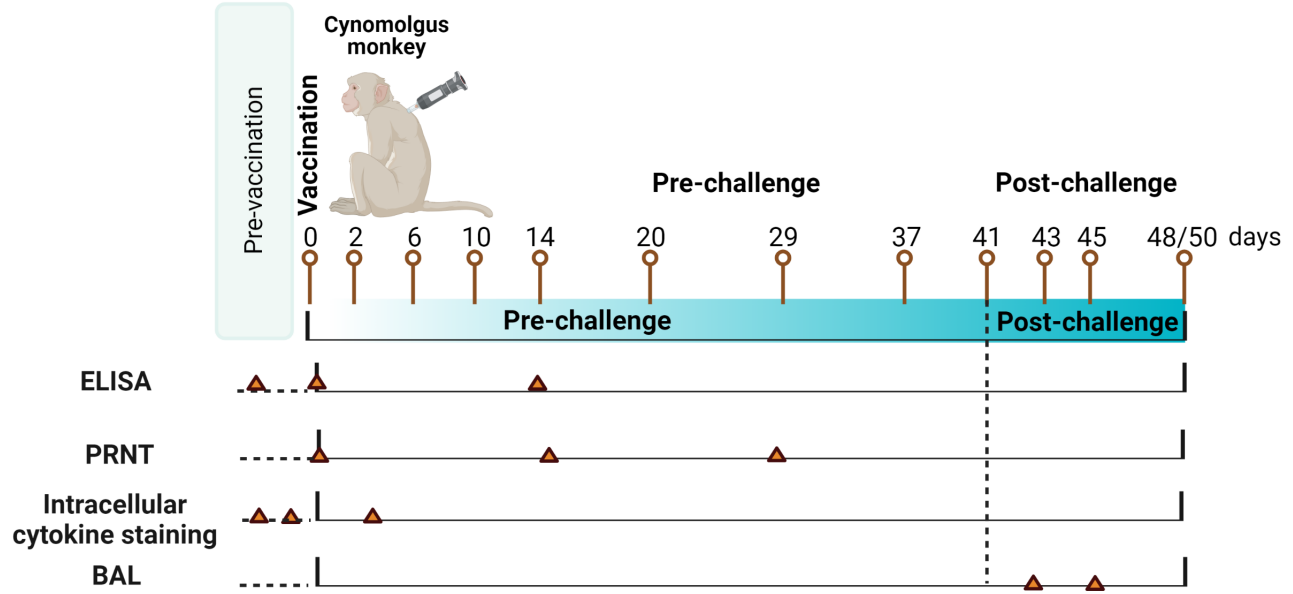
100% NHPs in TNX-1800 vaccinated group had no detectable SARS-CoV-2 genome

Virus Replication/Shedding: Tracheal Lavage



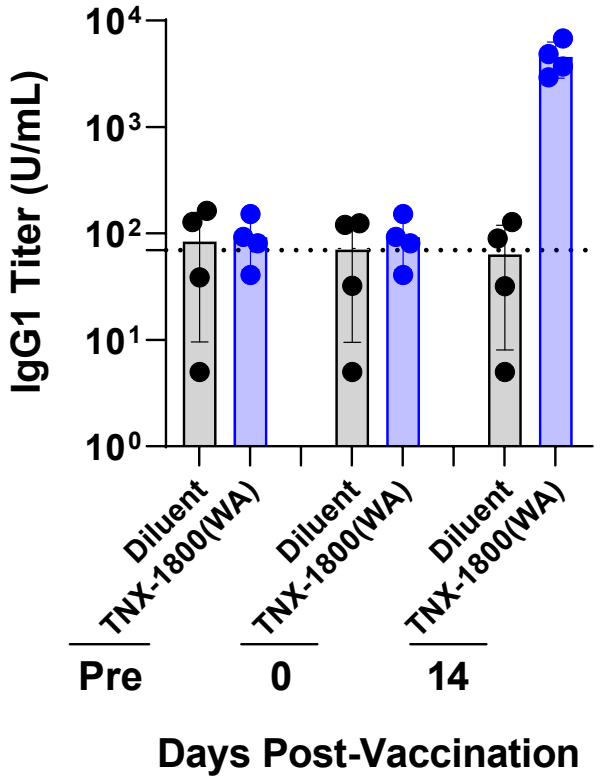
100% NHPs in TNX-1800 vaccinated group had no detectable SARS-CoV-2 genome

Preliminary Efficacy Study Design: Cynomolgus Macaques



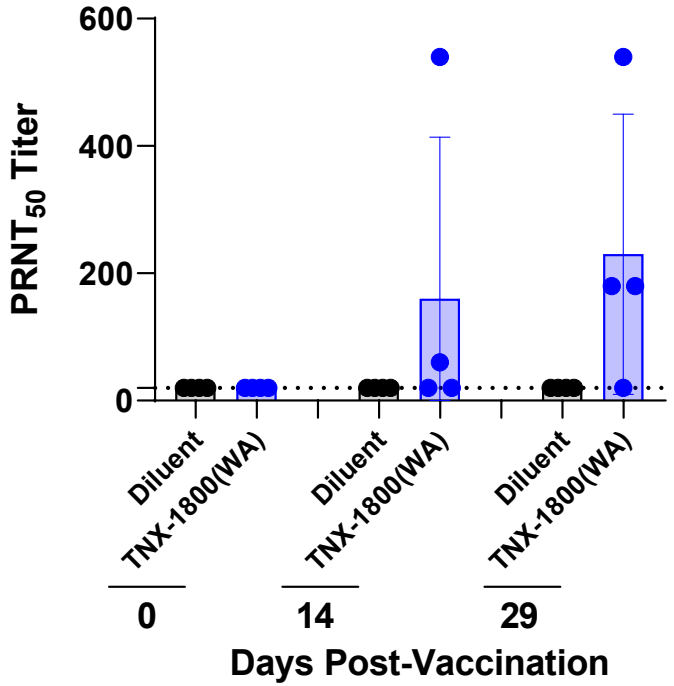
Vaccination					*Challenge*		
Group	Vaccine	N	Dose (Log_{10} PFU)	Route	*SARS-CoV-2 Challenge strain*	*Dose (Log_{10} PFU)*	*Route*
1	Diluent	4	Sham	Percutaneous	*USA-WA1/2020*	*5.0*	*IT/IN*
2	TNX-1800	4	6.1	Percutaneous	*USA-WA1/2020*	*5.0*	*IT/IN*

Immunogenicity: Total Anti-SARS-CoV-2 Spike Protein IgG1 Titer (ELISA)



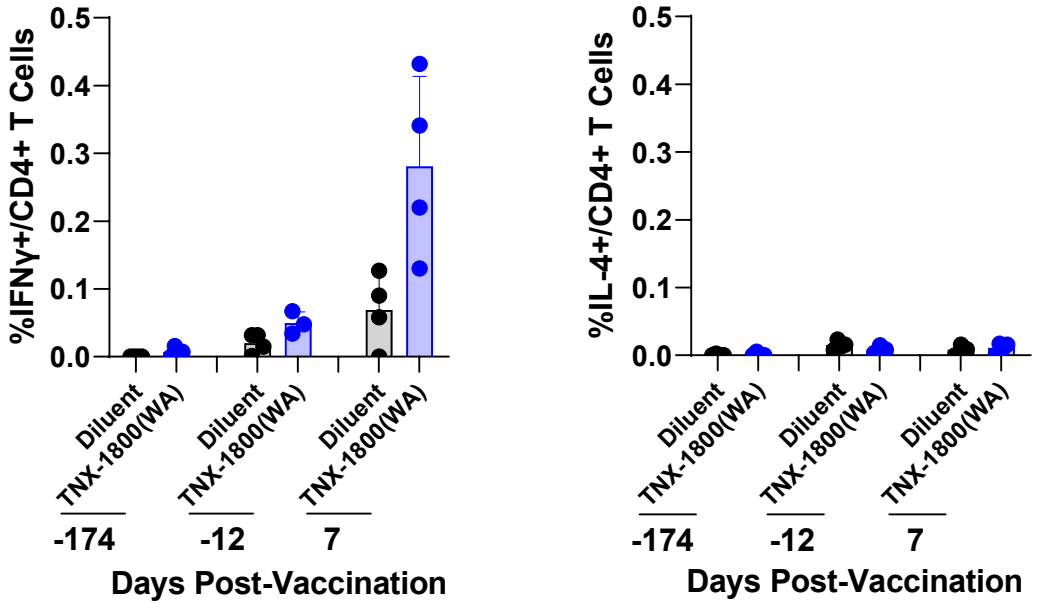
100% NHPs in TNX-1800 vaccinated group had IgG1 antibody response

Immunogenicity: Neutralizing Antibody ($PRNT_{50}$ Assay)



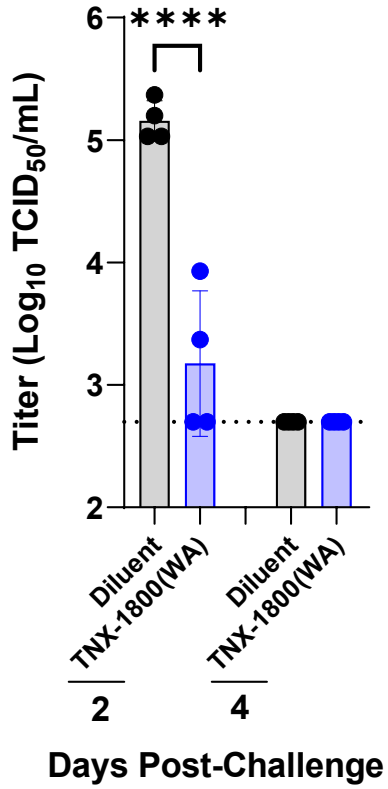
100% NHPs in TNX-1800 vaccinated group had neutralizing antibody response

Immunogenicity: Cell Mediated Response



100% NHPs in TNX-1800 vaccinated group had CD4⁺ T-cell/IFNγ (T_H1) response

Virus Replication/Shedding: Bronchoalveolar lavage (BAL) ($TCID_{50}$)



Infectious virus declined rapidly by ~100-fold in TNX-1800 vaccinated group

Conclusions

- TNX-1800 engineered to expressed heterologous antigen
  - "Proof of concept"
  - SARS-CoV-2 WA-2020 Spike protein
- 2 preliminary immunogenicity and 2 efficacy studies
  - Animal models: Hamsters, Rabbits, Cynomolgus and African green macaques
- A single dose of TNX-1800 vaccination was well tolerated
  - No severe adverse events following vaccination
  - Did not produce disseminated infection in any animal model

Conclusions

- TNX-1800 vaccination via route percutaneous was immunogenic
  - 100% response in all 4 animal models
  - Rapid generation of antibody response (Total IgG and/or neutralizing antibody)
  - Induced $CD4^+$ T-cell response
    - Responses were skewed to T_H1
- Efficacy studies in cynomolgus and African green macaques
  - Challenged with SARS-CoV-2 WA-2020
  - Virus shedding/replication was reduced by ~10 to 1,000-fold

Conclusions

- No longer continuing with clinical development of SARS-CoV-2 vaccine program
  1) New variants (e.g., XBB) appear to be boosting pre-existing immunity resulting in "herd immunity"
  2) Challenging regulatory hurdles for clinical evidence
- Additional vector development for heterologous genes from other pathogens underway:
  1) Additional insertion sites for stable expression
  2) Multivalency for additional heterologous antigens
  3) Additional routes of vaccination

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  - Siobhan Fogarty
  - Helen Stillwell
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