UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
Washington, D.C. 20549

FORM 8-K

CURRENT REPORT

Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934

Date of report (date of earliest event reported): March 10, 2025

TONIX PHARMACEUTICALS HOLDING CORP.

(Exact name of registrant as specified in its charter)

Nevada	**001-36019**	**26-1434750**
(State or Other Jurisdiction of Incorporation)	**(Commission File Number)**	**(IRS Employer Identification No.)**

26 Main Street, Chatham, New Jersey 07928
(Address of principal executive offices) (Zip Code)

Registrant's telephone number, including area code: (862) 904-8182

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions (see General Instruction A.2. below):

☐ Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)
☐ Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)
☐ Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))
☐ Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Securities registered pursuant to Section 12(b) of the Act:

Title of each class	Trading Symbol(s)	Name of each exchange on which registered
Common Stock	TNXP	The NASDAQ Capital Market

Indicate by check mark whether the registrant is an emerging growth company as defined in Rule 405 of the Securities Act of 1933 (§ 230.405 of this chapter) or Rule 12b-2 of the Securities Exchange Act of 1934 (§ 240.12b-2 of this chapter).

Emerging growth company ☐

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 13(a) of the Exchange Act. ☐

Item 7.01. Regulation FD Disclosure.

On March 10, 2025, Tonix Pharmaceuticals Holding Corp. (the "Company") announced it was awarded a grant for $50,000 from the Medical CBRN Defense Consortium ("MCDC") to support the development of its TNX-801 (recombinant horsepox virus, live vaccine) vaccine candidate for mpox and smallpox. A copy of the press release which discusses this matter is furnished hereto as Exhibit 99.01, and incorporated herein by reference

The Company updated its TNX-801 product candidate presentation, which it intends to place on its website and which may contain nonpublic information. A copy of the presentation is furnished hereto as Exhibit 99.02, and incorporated herein by reference

The information in this Item 7.01 of this Current Report on Form 8-K, including Exhibits 99.01 and 99.02 attached hereto, shall not be deemed "filed" for purposes of Section 18 of the United States Securities Exchange Act of 1934 (the "Exchange Act") or otherwise subject to the liabilities of that section, nor shall they be deemed incorporated by reference in any filing under the United States Securities Act of 1933 or the Exchange Act, except as shall be expressly set forth by specific reference in such a filing.

Item 8.01. Other Events.

On March 10, 2025, the Company announced it was awarded a grant for $50,000 from MCDC to support the development of its TNX-801 vaccine candidate for mpox and smallpox. The grant will allow Tonix to develop a commercialization plan for TNX-801.

Also on March 10, 2025, the Company disclosed new data demonstrating that (i) TNX-801 is up to 100,000 times less virulent than traditional live-virus smallpox vaccines in immunocompromised mice, (ii) can be delivered via alternative route of vaccination and (iii) provides durable (>6 months) protection to rabbits against a lethal rabbitpox challenge.

Forward- Looking Statements

This Current Report on Form 8-K contains certain forward-looking statements within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the

Securities Exchange Act of 1934 and Private Securities Litigation Reform Act, as amended, including those relating to the Company's product development, clinical trials, clinical and regulatory timelines, market opportunity, competitive position, possible or assumed future results of operations, business strategies, potential growth opportunities and other statement that are predictive in nature. These forward-looking statements are based on current expectations, estimates, forecasts and projections about the industry and markets in which we operate and management's current beliefs and assumptions.

These statements may be identified by the use of forward-looking expressions, including, but not limited to, "expect," "anticipate," "intend," "plan," "believe," "estimate," "potential," "predict," "project," "should," "would" and similar expressions and the negatives of those terms. These statements relate to future events or our financial performance and involve known and unknown risks, uncertainties, and other factors which may cause actual results, performance or achievements to be materially different from any future results, performance or achievements expressed or implied by the forward-looking statements. Such factors include those set forth in the Company's filings with the SEC. Prospective investors are cautioned not to place undue reliance on such forward-looking statements, which speak only as of the date of this press release. The Company undertakes no obligation to publicly update any forward-looking statement, whether as a result of new information, future events or otherwise.

Item 9.01. Financial Statements and Exhibits.

(d)

Exhibit No.	Description.
99.01	Press Release of the Company, March 10, 2025
99.02	TNX-801 Product Presentation
104	Cover Page Interactive Data File (embedded within the Inline XBRL document)

SIGNATURE

Pursuant to the requirement of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned thereunto duly authorized.

TONIX PHARMACEUTICALS HOLDING CORP.

Date: March 10, 2025

By: /s/ Bradley Saenger
Bradley Saenger
Chief Financial Officer

EXHIBIT 99.01

Tonix Pharmaceuticals Announces Grant by Medical CBRN Defense Consortium (MCDC) for Development of TNX-801, the Company's Single-Dose Mpox and Smallpox Vaccine Candidate

The World Health Organization (WHO) recently reaffirmed the spread of new clade Ib Mpox a public health emergency of international concern (PHEIC): second Mpox-related WHO PHEIC declaration in two years

Clade Ib Mpox cases detected in several countries in Central and Eastern Africa as well as China, Thailand, Singapore, India, England, parts of Europe and the Middle East, Canada and the United States

Tolerability of TNX-801 vaccination in immune-compromised animal models supports clinical development

CHATHAM, N.J., March 10, 2025 (GLOBE NEWSWIRE) -- Tonix Pharmaceuticals Holding Corp. (Nasdaq: TNXP) (Tonix or the Company), a fully-integrated biopharmaceutical company with marketed products and a pipeline of development candidates, today announced it has been awarded a grant from the Medical CBRN Defense Consortium (MCDC) to support the development of TNX-801 (recombinant horsepox virus, live vaccine). MCDC is a consortium of industrial, academic, and non-profit entities that supports the U.S. government in meeting military requirements for medical products to protect against chemical, biological, radiological and nuclear (CBRN) threats.TNX-801 is in development as an mpox and smallpox vaccine with the potential to be delivered via innovative alternative methods to improve patient compliance, ease of use and tolerability. The MCDC grant will allow for further comprehensive market analyses, target market identification and commercialization planning, including for both private and government markets.

"We are excited by the opportunity to collaborate with MCDC and are thankful for their support of our vaccine candidate," said Seth Lederman, M.D., President, and Chief Executive Officer of Tonix. "TNX-801 offers an appealing target product profile, requiring just a single dose for durable, long-term protection, with favorable shipping and storage requirements. With a significant global unmet need, TNX-801 is in a strong position to make a potential impact towards preventing mpox and controlling mpox epidemics."

TNX-801 is an attenuated live-virus vaccine based on synthesized horsepox that has been shown to provide single-dose immune protection against a monkeypox challenge with better tolerability than 20th century vaccinia live-virus vaccines in animals. TNX-801 has previously been shown to protect animals against lethal challenge with intratracheal clade I monkeypox virus.[1] An outbreak of clade I mpox was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) in August of 2024 and reaffirmed in February 2025.[2,3] Starting from an outbreak in the Democratic Republic of the Congo, clade I mpox has spread to sixteen Central African Countries and outside of Africa, including in China, Thailand, Singapore, India, England, parts of Europe and the Middle East, Canada and the United States.[4]

Tonix Pharmaceuticals Holding Corp.*

Tonix is a fully-integrated biopharmaceutical company focused on transforming therapies for pain management and vaccines for public health challenges. Tonix's development portfolio is focused on central nervous system (CNS) disorders. Tonix's priority is to advance TNX-102 SL, a product candidate for the management of fibromyalgia, for which an NDA was submitted based on two statistically significant Phase 3 studies for the management of fibromyalgia and for which a PDUFA (Prescription Drug User Fee act) goal date of August 15, 2025 has been assigned for a decision on marketing authorization. The FDA has also granted Fast Track designation to TNX-102 SL for the management of fibromyalgia. TNX-102 SL is also being developed to treat acute stress reaction and acute stress disorder under a Physician-Initiated IND at the University of North Carolina in the OASIS study funded by the U.S. Department of Defense (DoD). Tonix's CNS portfolio includes TNX-1300 (cocaine esterase), a biologic in Phase 2 development designed to treat cocaine intoxication that has FDA Breakthrough Therapy designation, and its development is supported by a grant from the National Institute on Drug Abuse. Tonix's immunology development portfolio consists of biologics to address organ transplant rejection, autoimmunity and cancer, including TNX-1500, which is an Fc-modified humanized monoclonal antibody targeting CD40-ligand (CD40L or CD154) being developed for the prevention of allograft rejection and for the treatment of autoimmune diseases. TNX-1500 has completed a positive Phase I trial. Tonix's infectious disease portfolio includes TNX-801, a vaccine in development for mpox and smallpox, as well as TNX-4200 for which Tonix has a contract with the U.S. DoD's Defense Threat Reduction Agency (DTRA) for up to $34 million over five years. TNX-4200 is a small molecule broad-spectrum antiviral agent targeting CD45 for the prevention or treatment of infections to improve the medical readiness of military personnel in biological threat environments. Tonix owns and operates a state-of-the art infectious disease research facility in Frederick, Md. Tonix Medicines, our commercial subsidiary, markets Zembrace® SymTouch® (sumatriptan injection) 3 mg and Tosymra® (sumatriptan nasal spray) 10 mg for the treatment of acute migraine with or without aura in adults.

* Tonix's product development candidates are investigational new drugs or biologics; their efficacy and safety have not been established and have not been approved for any indication.

This press release and further information about Tonix can be found at www.tonixpharma.com.

1 NoyceRS, et al. Viruses. 2023 Jan 26;15(2):356. Doi: 10.3390/v15020356. PMID: 36851570; PMCID: PMC9965234

2 WHO Press Release August 14, 2024. "WHO Director-General declares mpox outbrfeak a public health emergency of international concern". URL: www.who.int/news/item/14-08-2024-who-director-general-declares-mpox-outbreak-a-public-health-emergency-of-international-concern (accessed 8-15-24)

[3] McQuiston JH, et al. *U.S. Preparedness and Response to Increasing Clade I Mpox Cases in the Democratic Republic of the Congo* . 2024, MMWR Morbi Mortal Wkly Rep: United States. p. 435-440

[4] https://https://www.cdc.gov/mpox/situation-summary/

Forward Looking Statements

Certain statements in this press release are forward-looking within the meaning of 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,” “expect,” 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, risks related to the failure to obtain FDA clearances or approvals and noncompliance with FDA regulations; risks related to the failure to successfully market any of our products; 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. Tonix does not undertake an obligation to update or revise any forward-looking statement. 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 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. The information set forth herein speaks only as of the date thereof.

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Indication and Usage

Zembrace® SymTouch® (sumatriptan succinate) injection (Zembrace) and Tosymra® (sumatriptan) nasal spray are prescription medicines used to treat acute migraine headaches with or without aura in adults who have been diagnosed with migraine.

Zembrace and Tosymra are not used to prevent migraines. It is not known if Zembrace or Tosymra are safe and effective in children under 18 years of age.

Important Safety Information

Zembrace and Tosymra can cause serious side effects, including heart attack and other heart problems, which may lead to death. Stop use and get emergency help if you have any signs of a heart attack:

- discomfort in the center of your chest that lasts for more than a few minutes or goes away and comes back
- severe tightness, pain, pressure, or heaviness in your chest, throat, neck, or jaw
- pain or discomfort in your arms, back, neck, jaw or stomach
- shortness of breath with or without chest discomfort
- breaking out in a cold sweat
- nausea or vomiting
- feeling lightheaded

Zembrace and Tosymra are not for people with risk factors for heart disease (high blood pressure or cholesterol, smoking, overweight, diabetes, family history of heart disease) unless a heart exam shows no problem.

Do not use Zembrace or Tosymra if you have:

- history of heart problems
- narrowing of blood vessels to your legs, arms, stomach, or kidney (peripheral vascular disease)
- uncontrolled high blood pressure
- hemiplegic or basilar migraines. If you are not sure if you have these, ask your provider.
- had a stroke, transient ischemic attacks (TIAs), or problems with blood circulation
- severe liver problems
- taken any of the following medicines in the last 24 hours: almotriptan, eletriptan, frovatriptan, naratriptan, rizatriptan, ergotamines, or dihydroergotamine. Ask your provider for a list of these medicines if you are not sure.
- are taking certain antidepressants, known as monoamine oxidase (MAO)-A inhibitors or it has been 2 weeks or less since you stopped taking a MAO-A inhibitor. Ask your provider for a list of these medicines if you are not sure.
- an allergy to sumatriptan or any of the components of Zembrace or Tosymra

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Tell your provider about all of your medical conditions and medicines you take, including vitamins and supplements.

Zembrace and Tosymra can cause dizziness, weakness, or drowsiness. If so, do not drive a car, use machinery, or do anything where you need to be alert.

Zembrace and Tosymra may cause serious side effects including:

- changes in color or sensation in your fingers and toes
- sudden or severe stomach pain, stomach pain after meals, weight loss, nausea or vomiting, constipation or diarrhea, bloody diarrhea, fever
- cramping and pain in your legs or hips; feeling of heaviness or tightness in your leg muscles; burning or aching pain in your feet or toes while resting; numbness, tingling, or weakness in your legs; cold feeling or color changes in one or both legs or feet
- increased blood pressure including a sudden severe increase even if you have no history of high blood pressure
- medication overuse headaches from using migraine medicine for 10 or more days each month. If your headaches get worse, call your provider.
- serotonin syndrome, a rare but serious problem that can happen in people using Zembrace or Tosymra, especially when used with anti-depressant medicines called SSRIs or SNRIs. Call your provider right away if you have: mental changes such as seeing things that are not there (hallucinations), agitation, or coma; fast heartbeat; changes in blood pressure; high body temperature; tight muscles; or trouble walking.
- hives (itchy bumps); swelling of your tongue, mouth, or throat
- seizures even in people who have never had seizures before

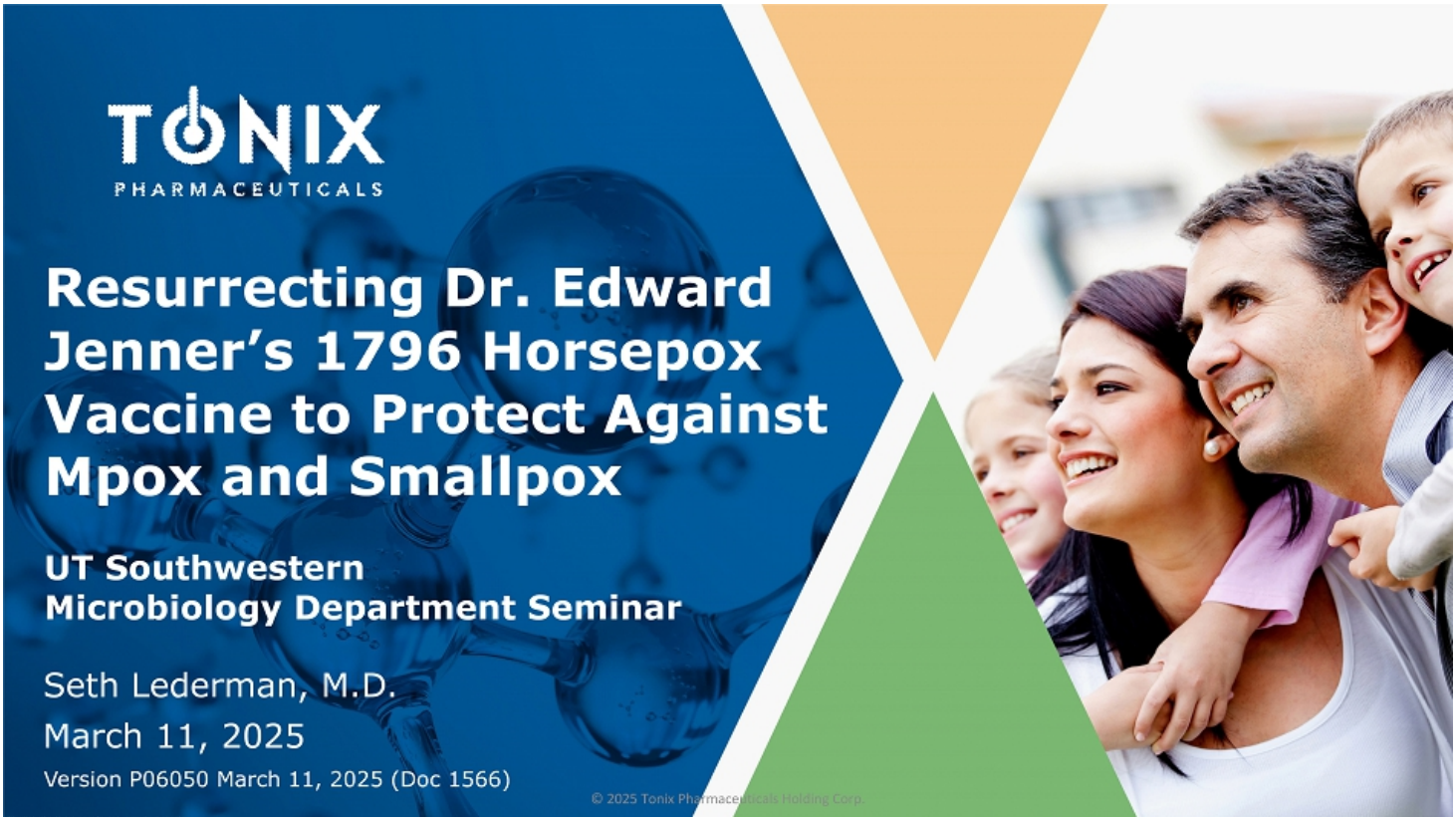
The most common side effects of Zembrace and Tosymra include: pain and redness at injection site (Zembrace only); tingling or numbness in your fingers or toes; dizziness; warm, hot, burning feeling to your face (flushing); discomfort or stiffness in your neck; feeling weak, drowsy, or tired; application site (nasal) reactions (Tosymra only) and throat irritation (Tosymra only).

Tell your provider if you have any side effect that bothers you or does not go away. These are not all the possible side effects of Zembrace and Tosymra. For more information, ask your provider.

This is the most important information to know about Zembrace and Tosymra but is not comprehensive. For more information, talk to your provider and read the Patient Information and Instructions for Use. You can also visit https://www.tonixpharma.com or call 1-888-869-7633.

You are encouraged to report adverse effects of prescription drugs to the FDA. Visit www.fda.gov/medwatch, or call 1-800-FDA-1088.

EXHIBIT 99.02



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.

Key Contributors

Tonix

Sina Bavari

Farooq Nasar

Scott Goebel

Zeil Rosenberg

Univ. of Alberta

Ryan Noyce

David Evans

In 1798, Edward Jenner Described *Vaccination* with the "Virus" that Causes Cow Pox for Preventing Smallpox

Jenner, E. (1798) "*The Inquiry*"

- Full title: "An Inquiry Into the Causes and Effects of the *Variolae Vaccinae*, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucestershire, and Known by the Name of the Cow Pox"

Cow Pox was a mild illness in humans that provided protection (later known as *immunity*)

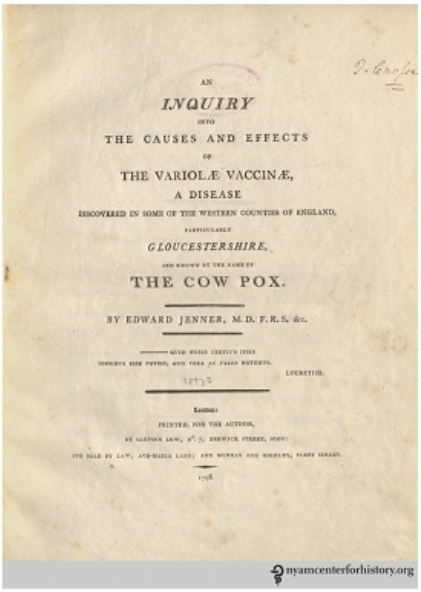
- "Cowpox" was the name of a disease in cows that could transfer to humans and cause sores

Jenner predicted eradication of smallpox

- In 1801, Jenner wrote "the annihilation of the smallpox, the most dreadful scourge of the human species, must be the final result of this practice."

Mpox benefit

- When vaccination for smallpox was widely practiced, mpox was kept out of the human population


AN
INQUIRY
INTO
THE CAUSES AND EFFECTS
OF
THE VARIOLÆ VACCINÆ,
A DISEASE
DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND,
PARTICULARLY
GLOUCESTERSHIRE,
AND KNOWN BY THE NAME OF
THE COW POX.
BY EDWARD JENNER, M.D. F.R.S. &c.
LUCRETIUS.
PRINTED, FOR THE AUTHOR,
1798.

Two Vaccines FDA-Approved for Smallpox and Mpox

Both are derived from Jenner's Vaccine from >200 years ago

ACAM2000 (live-virus vaccine) - Emergent

- Replicating - based on a clone of live-virus vaccinia (Dryvax®)
- Single-dose
- Provides durable protection – years or decades
- Tolerability concerns (myocarditis, pericarditis) limit widespread use[1]

Jynneos® (MVA) – Bavarian Nordic

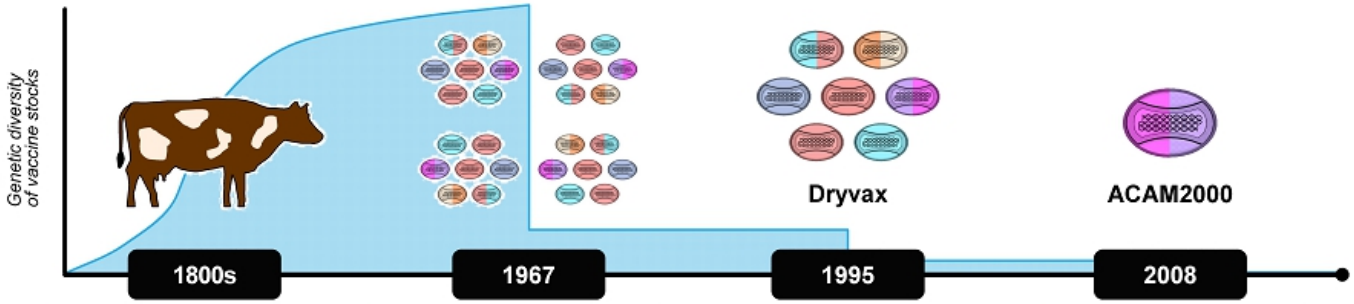
- Non-replicating – derived from passage in bird cells
- 2-dose regimen
- Durability of neutralization antibody titers being studied[2,3]
- Efficacy concerns in vaccination campaigns for mpox (relating to drop-outs)

Relative to historical accounts of Jenner's original vaccine:

- ACAM2000 appears have become more virulent
- MVA requires two doses and questions have been raised about durability of protection

[1]Engler RJM,, et al. (2015) A Prospective Study of the Incidence of Myocarditis/Pericarditis and New Onset Cardiac Symptoms following Smallpox and Influenza Vaccination. PLoS ONE 10(3)
[2]Zaeck LM, *Nat Med.* 2023 29(1):270-278. doi: 10.1038/s41591-022-02090
[3]Berens-Riha N, et al. *Euro Surveill.* 2022 27(48):2200894. doi: 10.2807/1560-7917.ES.2022.27.48.2200894.

Live Virus Smallpox Vaccines in the National Stockpile Are Derived From the NYCBH Strain



Propagation of Jenner's vaccinia in cows rapidly increases genetic diversity and results in many strains with varying properties[1,2]

WHO selects the NYCBH strain and 3 others for its eradication campaign, leading to a decrease in vaccine genetic diversity[3]

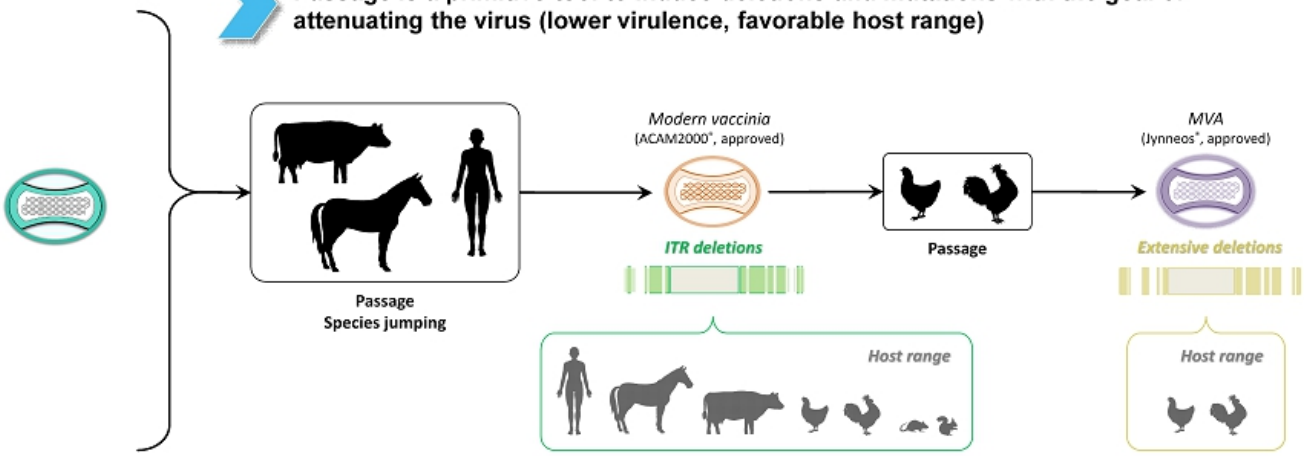
Dryvax, a vaccine swarm based on the NYCBH strain, is stockpiled in the US[4]

ACAM2000, a single virus selected from the Dryvax swarm, replaces Dryvax in the national stockpile[4,5]

[1]Noyce RS, et al. PLoS One. 2018;13(1):e0188453.
[2]The College of Physicians of Philadelphia. Accessed July 15, 2021. https://www.historyofvaccines.org
[3]Qin L, et al. J Virol. 2015;89(3):1809-1824.
[4]Nalca A, et al. Drug Des Devel Ther. 2010;4:71-79.
[5]Monath TP, et al. Int J Infect Dis. 2004;8 Suppl 2:S31-S44.

Passage or Zoonosis Leads to Genomic Deletions and New Host Range[1,2]

Passage is a primitive tool to induce deletions and mutations with the goal of attenuating the virus (lower virulence, favorable host range)



[1]Jacobs BL, et al. *Antiviral Res.* 2009;84(1):1-13.
[2]Belongia EA, et al. *Clin Med Res.* 2003;1(2):87-92.

U.S. Recognizes Smallpox Preparedness as a Priority National Stockpile Expansion is Recommended by Experts

BOX THE POX
REDUCING THE RISK OF SMALLPOX AND OTHER ORTHOPOXVIRUSES

A PLAN BY THE BIPARTISAN COMMISSION ON BIODEFENSE

February 2024

Smallpox and other orthopoxviruses pose significant threats to the United States and the world due to their potential for weaponization, accidental release, and vulnerability of populations who stopped routinely vaccinating against smallpox in the 1970s.[1]

(2-2) Smallpox vaccines that have improved safety across different population subgroups and are available as a single dose would support faster and more effective response to contain smallpox and other orthopoxvirus outbreaks. The development of novel smallpox vaccines using multi-vaccine platforms (i.e., use common vaccine vectors, manufacturing ingredients, and processes) would improve the capacity for rapid vaccine production in response to a smallpox event and reduce the need for stockpiling in the SNS at current levels.

Mpox and Smallpox Reports by U.S. Agencies & Institutions

Multiple recent statements by U.S. Agencies warning about smallpox and monkeypox[1-6]

U.S. National Academy of Sciences Consensus Report (March, 2024)[6]

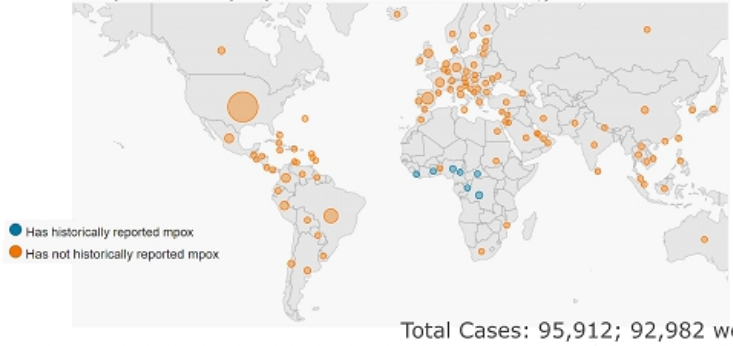
- *"Additionally, safer, single-dose vaccines and a diverse set of therapeutic options against smallpox would improve the U.S. readiness and response posture for immediate containment and long-term protection in a smallpox emergency.*
- *"Smallpox vaccines that have improved safety across different population subgroups and are available as a single dose would support faster and more effective response to contain smallpox and other orthopoxvirus outbreaks. The development of novel smallpox vaccines using multi-vaccine platforms (i.e., use common vaccine vectors, manufacturing ingredients, and processes) would improve the capacity for rapid vaccine production in response to a smallpox event and reduce the need for stockpiling in the SNS at current levels.*
- *"Given the lack of commercially available orthopoxvirus diagnostics, vaccines, and therapeutics, planning for logistics and supply chain management considerations is critical. Efforts could give consideration to developing plans to increase the number of smallpox vaccine and therapeutics manufacturers as well as optimizing current manufacturing capacities should they be needed in the shorter term."*

[1] Office of Science and Technology Policy (OSTP). American Pandemic Preparedness: Transforming Our Capabilities. September 2021
[2] National Biodefense Science Board (NBSB). Prioritization of Product Attribute Categories to Maximize Access for Next Generation COVID-19 Vaccines and Therapeutics. August 2023
[3] Office of Science and Technology Policy (OSTP). American Pandemic Preparedness: Transforming Our Capabilities. September 2021
[4] National Biodefense Science Board (NBSB). Prioritization of Product Attribute Categories to Maximize Access for Next Generation COVID-19 Vaccines and Therapeutics. August 2023
[5] BARDA Strategic Plan 2022-2026.
[6] U.S. National Academy of Sciences. March 28, 2024. "Consensus Study Report: Future State of Smallpox Medical Countermeasures."
https://nap.nationalacademies.org/catalog/27652/future-state-of-smallpox-medical-countermeasures

Mpox Outbreak 2022-23: Clade IIb: WHO Public Health Emergency Global Health Concern (PHEIC)

Risk of Spread and Lethality of Clade IIb

- Case Fatality Rate (CFR): 0.1% to 3.6%→Lower compared to Clade I
- Primarily spread through sexual contact among gay men outside Africa
- Rapid and significant spread beyond endemic regions → Over 90,000 cases reported in more than 100 countries by the end of 2022
- Systemic symptoms and rash leading to medical interventions in up to 40% of cases



Total Cases: 95,912; 92,982 were in locations that have not historically reported Mpox
Total Location: 118; 111 has not historically reported Mpox

Sources: WHO, European CDC, US CDC, and Ministries of Health
2022 U.S. Map and Case Count | Mpox | Poxvirus | CDC
WHO = World Health Organization
FDA = U.S. Food and Drug Administration

New Clade Ib Mpox Declared PHEIC* by WHO** in August 2024 and Reaffirmed in February 2025

Clade Ib - first wave in Democratic Republic of Congo (DRC)

- Spreads in households
- Affects children

Additional emerging mutation

- Potentially lower mortality
- Heterosexual transmission primarily in adults

2024 mpox epidemic in DRC has led to >20,000 cases by mid-August

- Spread to 12 countries in Africa, recently includes Kenya

Cases of Clade Ib in US occuring and many other countries outside of Africa

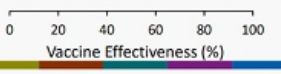
[1]Zaeck LM, *Nat Med.* 2023 29(1):270-278. doi: 10.1038/s41591-022-02090
[2]Berens-Riha N, et al. *Euro Surveill.* 2022 27(48):2200894. doi: 10.2807/1560-7917.ES.2022.27.48.2200894.
[3]August 30, 2024. Reuters. "US FDA approves Emergent's smallpox vaccine for people at high risk of mpox". https://www.msn.com/en-us/health/other/us-fda-approves-emergent-s-smallpox-vaccine-for-people-at-high-risk-of-mpox/

*Public Health Emergency of International Concern
**WHO = World Health Organization
***FDA = U.S. Food and Drug Administration

Non-replicating MVA Requires Two Doses: Drop-off in Protection from Mpox With Only One Dose

Vaccine effectiveness of JYNNEOS against mpox ranges from 36%–75% for 1-dose vaccination and 66%–89% for 2-dose vaccination

	Cases	Controls	Adjusted* VE (95% CI)
1-dose JYNNEOS			
Epic Cosmos case-control study	146	1000	36% (22–47)
Multi-jurisdictional case-control study	58	237	75% (61–84)
New York State case-control study	10	23	68% (25–86)
2-dose JYNNEOS			
Epic Cosmos case-control study	25	335	66% (47–78)
Multi-jurisdictional case-control study	14	122	86% (74–89)
New York State case-control study	2	19	89% (44–98)



ACIP Oct 25, 2023

U.S. Mpox Vaccine Coverage in High- Risk Groups (CDC)

1-dose: 38.8%
2-dose: 24.3%

37% Drop Out

Jenner was not Just "a Country Doctor"

***Linnean Society* member (1788) and *Royal Society*[1] fellow (1789) - PRIOR to Vaccination**

Jenner was the first and favorite student of John Hunter (1728-1793)

- –Hunter ran a private "medical school" in London
  - Surgeon / anatomist / variolator – Product of "The Scottish Enlightenment"
    - Grave robber / Body Snatcher
    - Hunter's school was based in his house in Leicester Square
  - Model for Robert Louis Stevenson's "*Strange Case of Dr. Jekyll and Mr. Hyde*" (1886)
    - Two separate entrances—one leading to his residence and the other to his dissecting rooms and museum

Jenner was involved in a semi-systematic search for improved "material" for "variolation" (inoculation with live smallpox)

- –Jenner was neither passive, nor "lucky" – he tried more than once
- –Jenner was one of several contemporaries who were searching among variola lesions and animal "sores" for improved variolation technology
  - Variola innovators: Thomas Dimsdale, John Hunter (Jenner's mentor), Jan Ingenhousz (who also discovered photosynthesis)
  - Other "cowpox" observers: Giovanni Maria Lancisi, John Fewster[2], Peter Plett, Benjamin Jesty, etc.
  - Related technologies: Pearl Pox, which caused "Milkers Nodules" – may have been parapoxvirus
- –Jenner's "laboratory" was a community with periodic outbreaks of smallpox, cowpox and horsepox

[1]Corresponded with Sir Joseph Banks, who became President of the Royal Society in 1778
[2]Fewster, John (1765). *Cow Pox and Its Ability to Prevent Smallpox* (Medical Society of London, unpublished paper)

Edward Jenner Successfully Used *Vaccination* to Protect Against Smallpox

- Jenner "vaccinated" healthy individuals with material from the lesions, which he called "vaccine" (from *vacca*, Latin for "cow")
- The pustule matter from "cow pox" sores on a milkmaid's hands; conferred protection against future challenges with smallpox virus inoculation
- Jenner suspected that the agent ("infectious principle") causing cow pox, which he called **vaccinia** originated in horses and had been transferred from horses to cows' udders by the hands of farriers

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

Edward Jenner Successfully Used *Vaccination* to Protect Against Smallpox: *Virus* came from Horses

Jenner "vaccinated" healthy individuals with "vaccine"

- From *vacca*, Latin for "cow"

The material from "cow pox" sores conferred protection against smallpox virus

- Originally came from a milkmaid's hands;

Jenner suspected that the virus originated in horses

- He observed that the virus had been transferred from horses to cows' udders by the hands of farriers

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

First Live Virus Vaccine: Edward Jenner's *Inquiry*[1] (1798) – 1/2

"There is a disease to which the **Horse** from his state of domestication is frequently subject. The Farriers have termed it *the Grease*. It is an inflammation and swelling in the heel, from which issues matter[2] possessing properties of a very peculiar kind, which seems capable of generating a disease in the Human Body (after it has undergone the modification[3] I shall presently speak of), which bears so strong a resemblance to the Small Pox, that I think it highly probable it may be the source of that disease."

[1]Jenner, E. "An Inquiry Into the Causes and Effects of the *Variolae Vaccinae*, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucestershire, and Known by the Name of the Cow Pox (p 2-3.)
[2]Vaccine virus
[3]Passage in cows

First Live Virus Vaccine: Edward Jenner's *Inquiry*[1] (1798) – 2/2

"In this Dairy Country a great number of Cows are kept, and the office of milking is performed indiscriminately by Men and Maid Servants. One of the former having been appointed to apply dressings to the heels of a **Horse** affected with *the Grease*, and not paying due attention to cleanliness, incautiously bears his part in milking the Cows, with some particles of the infectious matter adhering to his fingers. When this is the case, it commonly happens that a disease is communicated to the Cows, and from the Cows to the Dairy-maids, which spreads through the farm until most of the cattle and domestics feel its unpleasant consequences. The disease has obtained the name of the *Cow Pox*."

[1]Jenner, E. "An Inquiry Into the Causes and Effects of the *Variolae Vaccinae*, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucestershire, and Known by the Name of the Cow Pox (p 3.)

Loy's "*Account of some experiments*[1] (1801)

"This fact induces me to suspect, that two kinds of Grease exist, differing from each other in the power of giving disease to the human or brute animal: and there is another circumstance which renders this supposition probable. The **horses** that communicated the infection to their dressers, were affected with a general, as well as a topical, disease. The animals, at the commencement of their disease, were evidently in a feverish state, from which they were relived as soon as the complaint appeared at their heels, and an eruption upon their skin. The **horse**, too, from whom the infectious matter was procured for inoculation, had a considerable indisposition, previous to the disease at his heels, which was attended, as in the others, with an eruption over the greatest part of his body: but those that did not communicate the diseases at all, had a local affection only."

[1]Loy JG. An account of some experiments on the origin of the cow-pox: Whitby; 1801. (p 20-21.)

Equination[1]: Use of Smallpox Vaccines Directly from Horse Lesions (Without Passage Through Cows)

Both Jenner and Loy used vaccine from horses; subsequently "Equination" was used in Europe in parallel with "vaccination"

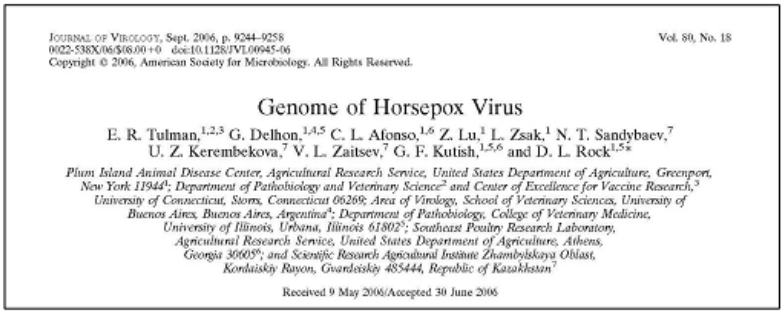
- –Jenner believed that his "cowpox" or "vaccinia" came from horses with "Grease"

Horsepox isolated from a sick horse in Mongolia in 1976

- –Like many other poxviruses, natural host is likely rodents (mice or voles)
- –No cases reported in >30 years, some believe it to be extinct; eliminated through improved animal husbandry

[1]Esparza J, Schrick L, Damaso CR, Nitsche A. Equination (inoculation of horsepox): An early alternative to vaccination (inoculation of cowpox) and the potential role of horsepox virus in the origin of the smallpox vaccine. *Vaccine*. 2017 Dec 19;35(52):7222-7230. doi: 10.1016/j.vaccine.2017.11.003. Epub 2017 Nov 11. Review. PMID:29137821

2006 Sequence and Analysis of the Horsepox Genome[1]



JOURNAL OF VIROLOGY, Sept. 2006, p. 9244–9258 Vol. 80, No. 18
0022-538X/06/$08.00+0 doi:10.1128/JVI.00945-06

Genome of Horsepox Virus

E. R. Tulman,[1,2,3] G. Delhon,[1,4,5] C. L. Afonso,[1,6] Z. Lu,[1] L. Zsak,[1] N. T. Sandybaev,[7] U. Z. Kerembekova,[7] V. L. Zaitsev,[7] G. F. Kutish,[1,5,6] and D. L. Rock[1,5]*

Plum Island Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Greenport, New York 11944[1]; Department of Pathobiology and Veterinary Science[2] and Center of Excellence for Vaccine Research,[5] University of Connecticut, Storrs, Connecticut 06269; Area of Virology, School of Veterinary Sciences, University of Buenos Aires, Buenos Aires, Argentina[4]; Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, Illinois 61802[3]; Southeast Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Athens, Georgia 30605[6]; and Scientific Research Agricultural Institute Zhambylskaya Oblast, Kordaiskiy Rayon, Gvardeiskiy 485444, Republic of Kazakhstan[7]

Received 9 May 2006/Accepted 30 June 2006

"It is likely that a once naturally circulating but now rare VACV-like virus(s) from which current strains are derived was introduced as a vaccine virus, and the agent of horsepox has been surmised as a likely candidate (Baxby, D 1981[2]). Indeed, apparently Edward Jenner believed that his vaccine originated from the "grease" infection found in the heels of horses, and the use of horse-derived material for use as vaccines is documented (Baxby, *ibid.*, Fenner F, 1989[3])."

[1]Tulman ER, et al. 2006. Genome of horsepox virus. *J Virol* 80:9244–9258.
[2] Baxby, D. 1981. Jenner's smallpox vaccine: the riddle of vaccinia virus and its origin. Heinemann Educational Books Ltd., London, United Kingdom.
[3] Fenner, F., R. Wittek, and K. Dumbell. 1989. The orthopoxviruses. Academic Press, Inc., San Diego, Calif.

2015 Genetic Analysis of Vaccinia Vaccines: Horsepox-like Virus Ancestor?[1]

February 2015 Volume 89 Number 3 Journal of Virology jvi.asm.org 1809

JVI Journals.ASM.org

Evolution of and Evolutionary Relationships between Extant Vaccinia Virus Strains

Li Qin,* Nicole Favis, Jakub Famulski,* David H. Evans

Department of Medical Microbiology & Immunology and Li Ka Shing Institute of Virology, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada

"The biological origin of VACV is uncertain, although it has been suggested that a horsepox-like virus was an ancestor, even though a surviving horsepox virus (HPXV) genome harbors many extra genes (Tulman ER, 2006[2]). This hypothesis is supported by Jenner's report that he obtained his later inocula from an infection in horses called "grease" (Baxby D, 1977[3])"

[1]Qin, L., Favis, N., Famulski, J. & Evans, D. H. Evolution of and evolutionary relationships between extant vaccinia virus strains. *J. Virol.* **89**, 1809–1824 (2015)
[2]Tulman ER, et al. 2006. Genome of horsepox virus. *J Virol* 80:9244–9258.
[3]Baxby D. 1977. The origins of vaccinia virus. J Infect Dis 136:453– 455. http://dx.doi.org/10.1093/infdis/136.3.453.

David Evans: Speciation and Gene Loss in Vaccinia

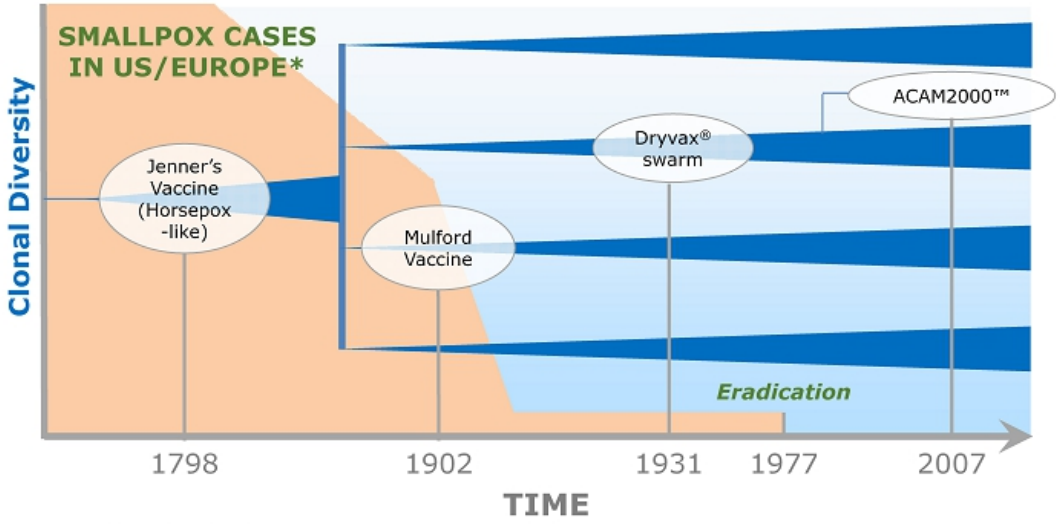
Evans, "...the process of speciation appears to be associated with gene loss."[1]

–"**Relationship between DPP25 and horsepox virus.** An important aspect of poxvirus evolutionary modeling concerns the hypothesis that as viruses spread into new biological niches, the process of speciation appears to be associated with gene loss (3). If this is true, then the simplest evolutionary scheme would involve a DPP25-like virus evolving from an even larger virus. Horsepox virus (HPXV) is the largest known example of what is still clearly a vaccinia virus, if one defines this assignment based upon a relationship supported by phylogenetic trees, and perhaps retains some resemblance to a hypothetical common ancestor.

[1]Qin, L., Favis, N., Famulski, J. & Evans, D. H. Evolution of and evolutionary relationships between extant vaccinia virus strains. *J. Virol.* **89**, 1809–1824 (2015)

Proposed Evolution of Vaccinia Vaccines

Relationship to Smallpox Incidence and Eradication



*Rough approximation (not data derived)

23

Synthesis of Horsepox (HPXV, TNX-801) 2018[1]

PLOS ONE

RESEARCH ARTICLE

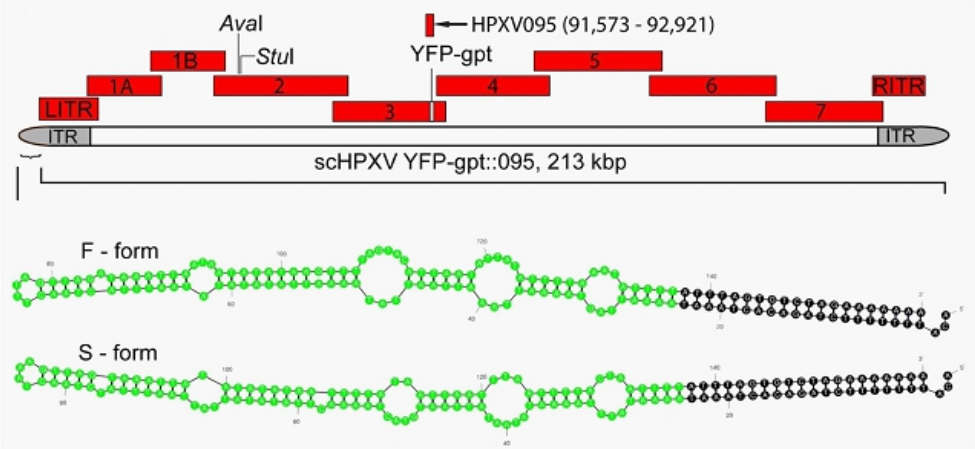
Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments

Ryan S. Noyce[1], Seth Lederman[2], David H. Evans[1]*

1 Department of Medical Microbiology & Immunology and Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada, **2** Tonix Pharmaceuticals, Inc., New York, New York, United States of America

[1]Noyce RS, Lederman S, Evans DH. *PLoS One*. 2018 Jan 19;13(1):e0188453. doi: 10.1371/journal.pone.0188453. PMID: 29351298; PMCID: PMC5774680.

Genome Assembly (212 kbp): TNX-801 Core Genome is Based on HPXV Strain MNR-76[1,2]



Sequence: GenBank entry DQ792504; DNA: GeneArt

[1]Noyce, RS, Lederman S, Evans DH. PLoS ONE. 2018; 13(1): e0188453
https://doi.org/10.1371/journal.pone.0188453
[2]Tulman ER, et al. Genome of horsepox virus, *J Virol*; 2006 80(18):9244-58.PMID:16940536

TNX-801 (Live HPXV for Percutaneous Administration)

Vaccine based on sequence of isolated horsepox (HPXV) clone[1]

- –Synthesized[2] since 1976 isolate was not available outside of the U.S. Centers for Disease Control and Prevention (CDC)
- –No new gene elements
- –Coding sequence identical to HPXV

Small plaque size in culture

- –Appears identical to U.S. CDC publication of 1976 horsepox isolate[3]

Question: will "horsepox" perform as a vaccine similar to "Jenner's vaccinia" and 20th Century vaccinia vaccines?

- –Need to evaluate tolerability and activity in animal models

[1]Tulman ER, et al. *J Virol*. 2006 80(18):9244-58.PMID:16940536
[2]Noyce RS, et al.. *PLoS One*. 2018 Jan 19;13(1):e0188453
[3]Trindade GS, et al. *Viruses* 2016 Dec 10;8(12). pii: E328. PMID:27973399 PMCID: 10.3390/v8120328

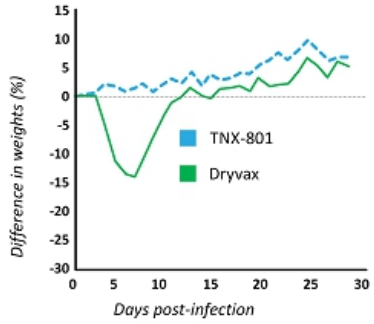
Vaccination with TNX-801 (rHPXV): Immunity with Low Reactogenicity (*i.e.*, Better Tolerability)

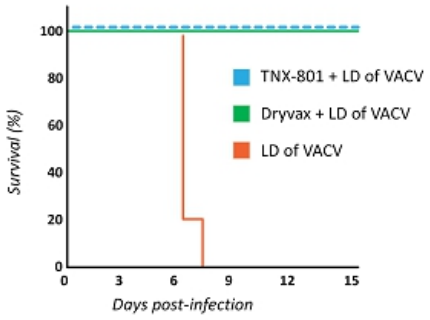
Efficacy and safety of TNX-801 compared to Dryvax ("circa 1960 vaccinia" strain)[1]:

–Mice (5 per group) infected with Dryvax lost up to 15% of their body weight because of illness induced by the vaccine, but mice infected with TNX-801 did not experience any weight loss or illness

–TNX-801 protected mice from a lethal dose (LD) of vaccinia (VACV), like Dryvax

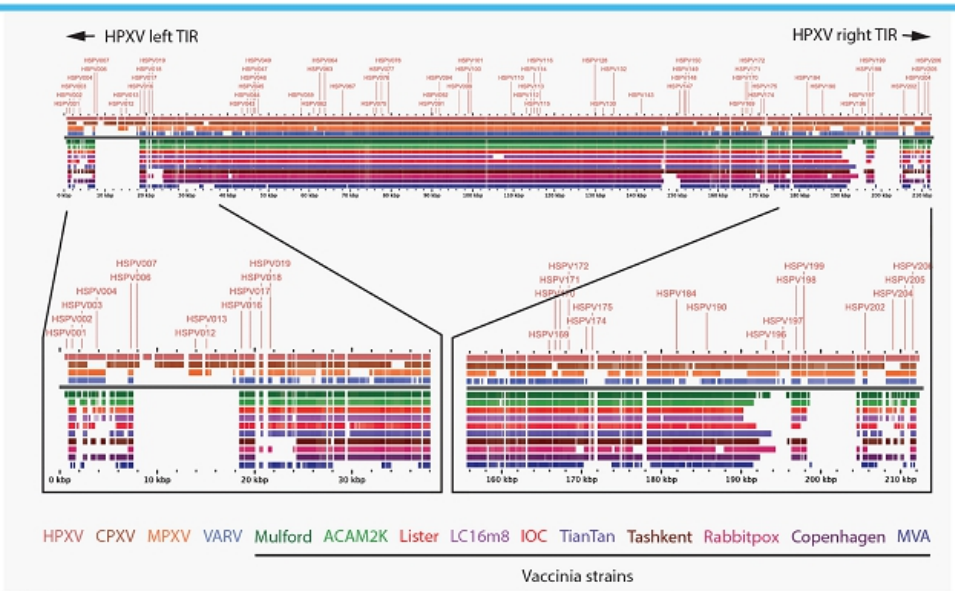
–TNX-801 may be safer (less reactogenic) than "circa 1960 Vaccinia" vaccines without sacrificing immune protection (efficacy)





[1]Noyce RS, et al.. Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. *PLoS One*. 2018 Jan 19;13(1):e0188453.

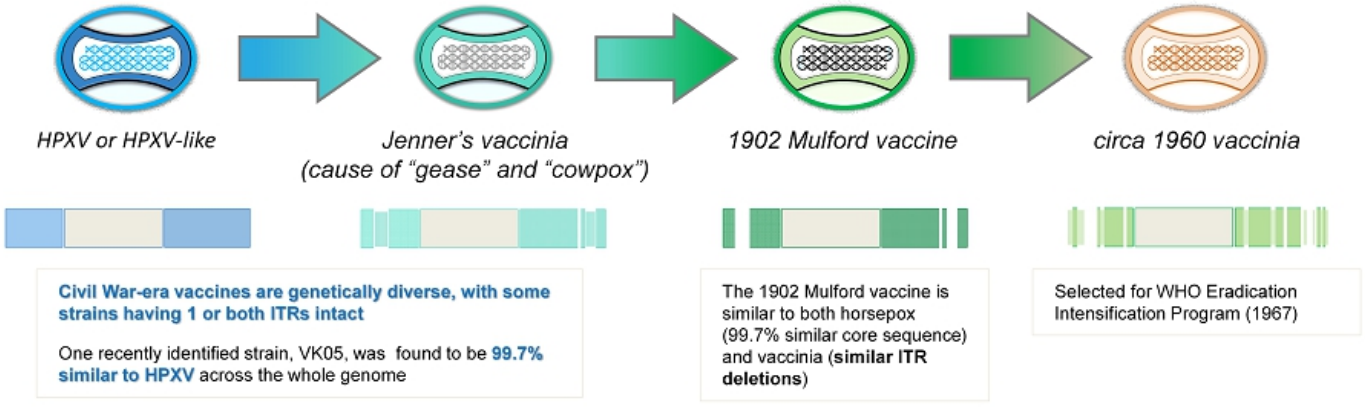
Horsepox Compared to Cowpox and Vaccinia Strains[1] Consistent with Near "Primordial" Strain Status



[1]Evans, D. U. of Alberta (2018) with permission

Evolution of the Vaccinia Genome

Recent studies (particularly from José Esparza & colleagues) demonstrate that horsepox and horsepox-like viruses were used as smallpox vaccines in the 1800s[1-3]



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% similar to HPXV** across the whole genome

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

Selected for WHO Eradication Intensification Program (1967)

[1]Schrick L, et al. *N Engl J Med.* 2017;377(15):1491-1492
[2]Duggan AT, et al. Genome Biol. 2020;21(1):175.
[3]Brinkmann A, et al. Genome Biol. 2020;21(1):286.

Horsepox: Relationship to Jenner's Vaccinia

Horsepox environmental isolate sequenced in 2006 shares a common ancestor with vaccinia and could be considered a strain of vaccinia

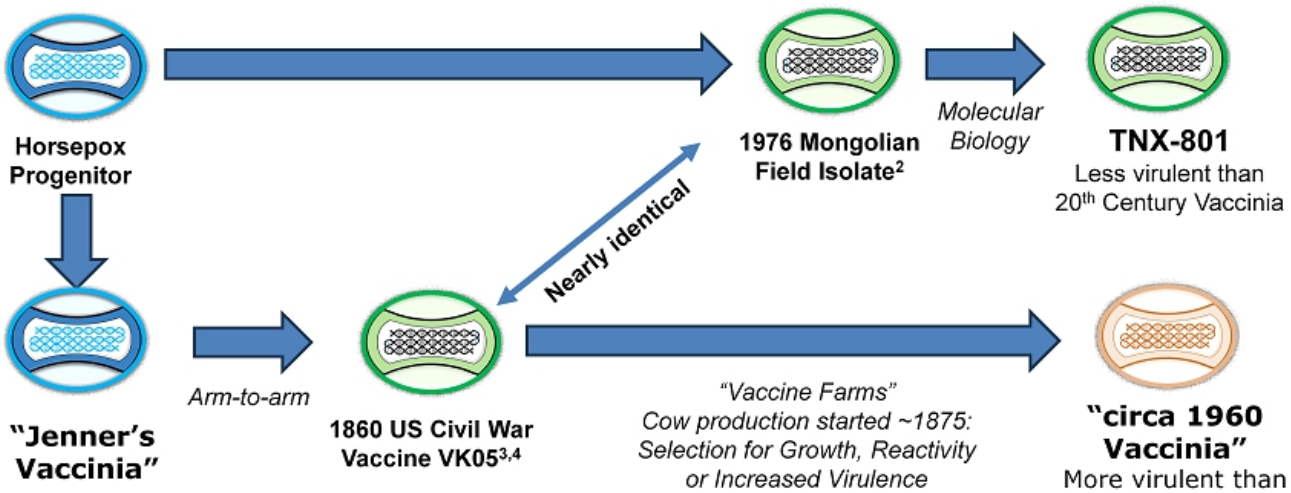
- –Similar to cowpox with "intact" inverted terminal repeats (ITRs) – could be considered a primordial strain of vaccinia
- –TNX-801 has strong homology in **core** with Mulford 1902 vaccinee[1]
- –TNX-801 has 99.7% colinear identity with "**circa 1860 vaccinia**" smallpox vaccine VK05, **including the LTRs/ITRs** that contain host control elements[2,3]

Genetic analysis of early vaccines indicates that "horsepox" is closely related to Edward Jenner's vaccinia from 1796

- –Strong evidence linking a horsepox-like virus as progenitor to circa 1960 vaccinia
- –circa 1960 "vaccinia" evolved during the 220 years it was propagated by primitive methods – Propagated for over 120 years before "viruses" were characterized
- –**Selected for reactogenicity and growth (replication)**

[1]Schrick, L. et al An Early American Smallpox Vaccine Based on Horsepox *N Engl J Med* 2017; 377:1491
[2]Tulman ER, et al. Genome of horsepox virus. *J Virol*; 2006 80(18):9244-58.PMID:16940536
[3]Brinkmann A et al, *Genome Biology* 2020; 21:286 https://doi.org/10.1186/s13059-020-02202-0

Deduced Relationship of Horsepox with "Jenner's Vaccinia" and "circa 1960 Vaccinia" Vaccines



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[3]Trindade GS, et al. *Viruses* 2016 Dec 10;8(12). pii: E328. PMID:27973399 PMCID: 10.3390/v8120328

TNX-801 Immunogenicity and Efficacy in NHPs - 2023

viruses

MDPI

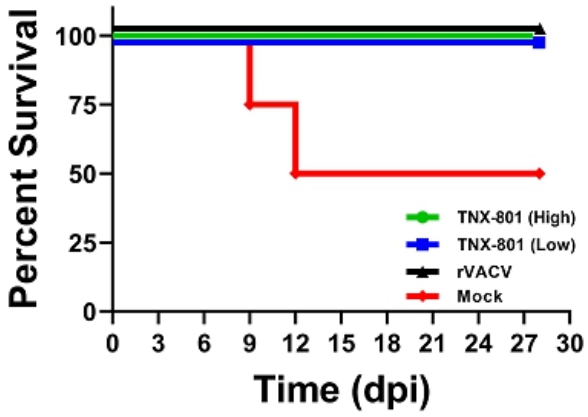
Article

Single Dose of Recombinant Chimeric Horsepox Virus (TNX-801) Vaccination Protects Macaques from Lethal Monkeypox Challenge

Ryan S. Noyce [1], Landon W. Westfall [2,†], Siobhan Fogarty [3], Karen Gilbert [2], Onesmo Mpanju [4], Helen Stillwell [3,‡], José Esparza [5], Bruce Daugherty [3], Fusataka Koide [2], David H. Evans [1] and Seth Lederman [3,*]

Noyce RS, et al. *Viruses*. 2023 Jan 26;15(2):356. doi: 10.3390/v15020356. PMID: 36851570; PMCID: PMC9965234.

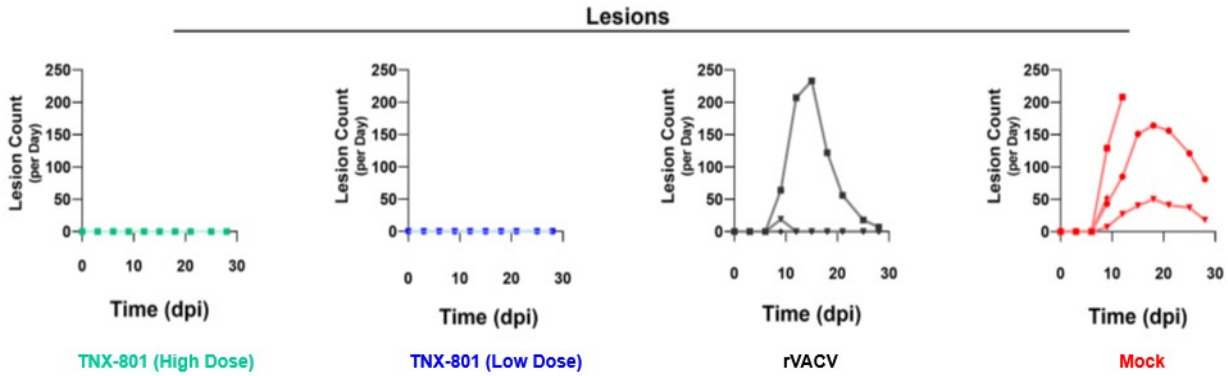
Survival: 100% of TNX-801 Vaccinated NHPs Survived Lethal MPXV Clade 1 Intratracheal Challenge



No deaths in TNX-801 vaccinated groups

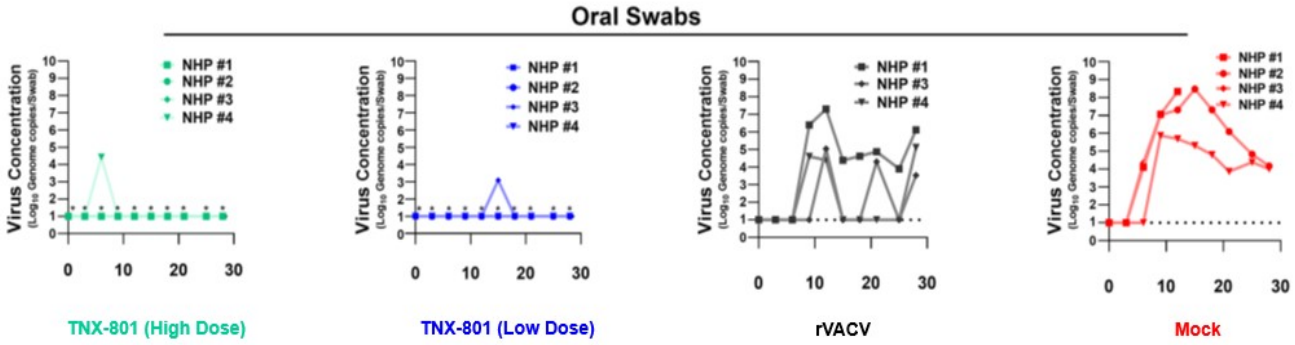
Noyce *et al*. Viruses. 2023 Jan 26;15(2):356.

TNX-801 Vaccination/MPXV Clade 1 Challenge: No Lesions Were Observed After TNX-801 Vaccination



Noyce *et al*. Viruses. 2023 Jan 26;15(2):356.

TNX-801 Vaccination: Minimal MPXV Virus Shedding



Potential to Reduce Forward Transmission

Noyce *et al*. Viruses. 2023 Jan 26;15(2):356.

Conclusions from NHP MPXV Challenge Study

A single dose of TNX-801 (horsepox) vaccination was well tolerated
- No severe adverse events
- Tolerability compares favorably to ACAM2000 – recently approved by US FDA for mpox[1]

TNX-801 vaccination via traditional route (scarification) was immunogenic ("take")

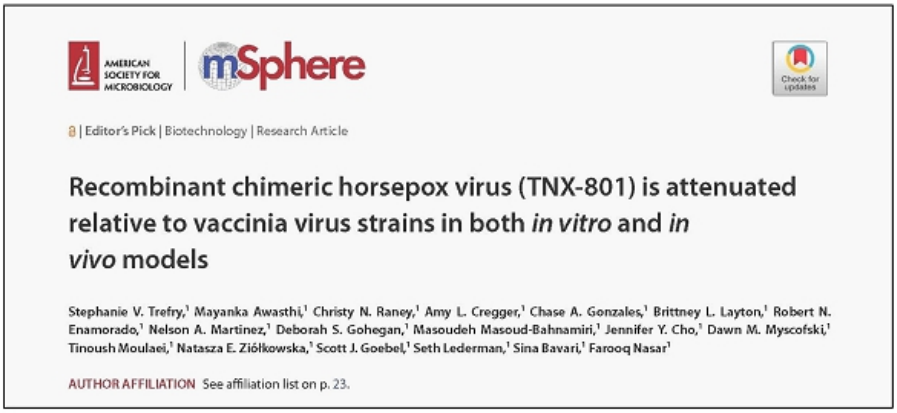
All NHPs (TNX-801 and rVACV vaccinated) survived lethal challenge

No clinical disease was observed (lesions)

Provided strong protection against virus shedding, viremia, and weight loss
- Activity compares favorably to MVA (non-replicating)[2] vaccinia or recent mRNA vaccine[3]

[1]August 30, 2024. Reuters. "US FDA approves Emergent's smallpox vaccine for people at high risk of mpox". https://www.msn.com/en-us/health/other/us-fda-approves-emergent-s-smallpox-vaccine-for-people-at-high-risk-of-mpox/
[2]Zaeck LM, et al. Low levels of monkeypox virus-neutralizing antibodies after MVA-BN vaccination in healthy individuals. Nat Med. 2023 Jan;29(1):270-278. doi: 10.1038/s41591-022-02090-w. Epub 2022 Oct 18. PMID: 36257333; PMCID: PMC9873555.
[3]Mucker E et al., Comparison of protection against mpox following mRNA or modified vaccinia Ankara vaccination in nonhuman primates, Cell (2024), https://doi.org/10.1016/j.cell.2024.08.043

TNX-801 in Primary Cell Lines and Immunocompromised Mice – 2024 *(mSphere)*



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8 | Editor's Pick | Biotechnology | Research Article

Recombinant chimeric horsepox virus (TNX-801) is attenuated relative to vaccinia virus strains in both *in vitro* and *in vivo* models

Stephanie V. Trefry,[1] Mayanka Awasthi,[1] Christy N. Raney,[1] Amy L. Cregger,[1] Chase A. Gonzales,[1] Brittney L. Layton,[1] Robert N. Enamorado,[1] Nelson A. Martinez,[1] Deborah S. Gohegan,[1] Masoudeh Masoud-Bahnamiri,[1] Jennifer Y. Cho,[1] Dawn M. Myscofski,[1] Tinoush Moulaei,[1] Natasza E. Ziółkowska,[1] Scott J. Goebel,[1] Seth Lederman,[1] Sina Bavari,[1] Farooq Nasar[1]

AUTHOR AFFILIATION See affiliation list on p. 23.

Trefry SV, et al. *mSphere*. 2024 Dec 19;9(12):e0026524. doi: 10.1128/msphere.00265-24. Epub 2024 Nov 13. PMID: 39535212; PMCID: PMC11656774.

TNX-801 has Reduced Virulence Relative to "circa 1960 Vaccinia"

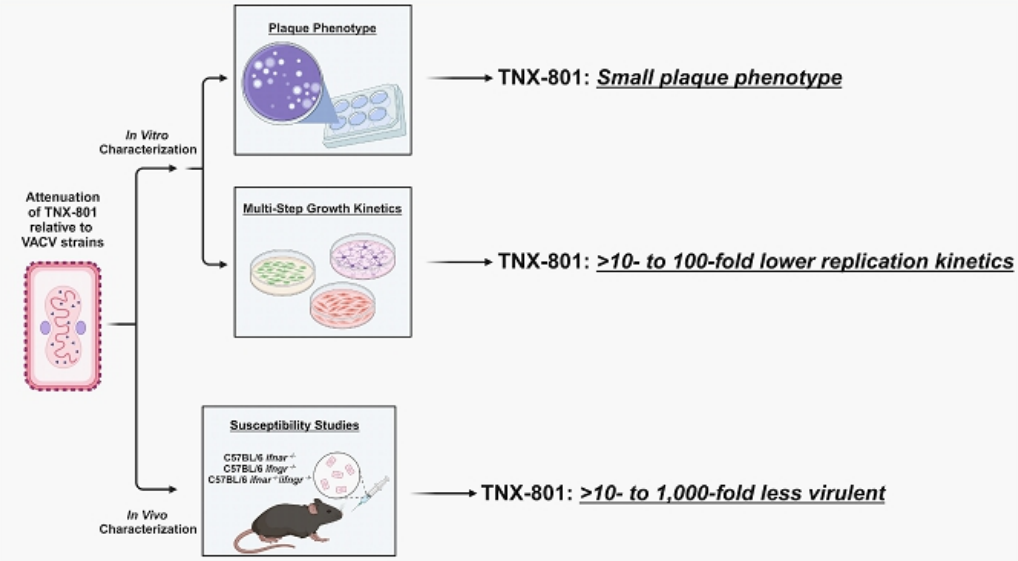
Comparisons *in vitro:*

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

Comparisons *in vivo*:

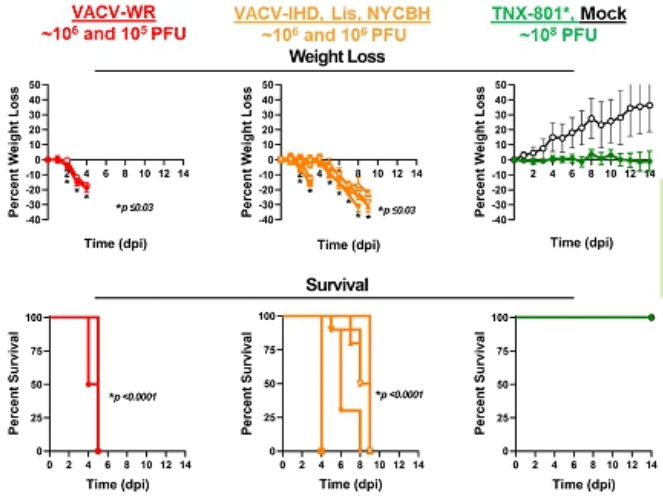
1) Assessed TNX-801 attenuation in immunocompromised murine models (C57BL/6 *ifnar*$^{-/-}$ *and* C57BL/6 *ifnar*$^{-/-}$/*ifngr*$^{-/-}$) :
   - TNX-801 is >100- to 1,000-fold less virulent than VACV strains
   - TNX-801 is indistinguishable from mock treated animals in immunocompromised model

Conclusion: TNX-801 is 10-to-1000-fold Less Virulent than 20th Century Vaccinia (VACV)



TNX-801 Lacks Lethality Associated With Older Smallpox Vaccine Strains (Lister, NYC Board of health) in Double KO IFN-αR$^{-/-}$ and IFN-γR$^{-/-}$ Mice

(1 of 2)



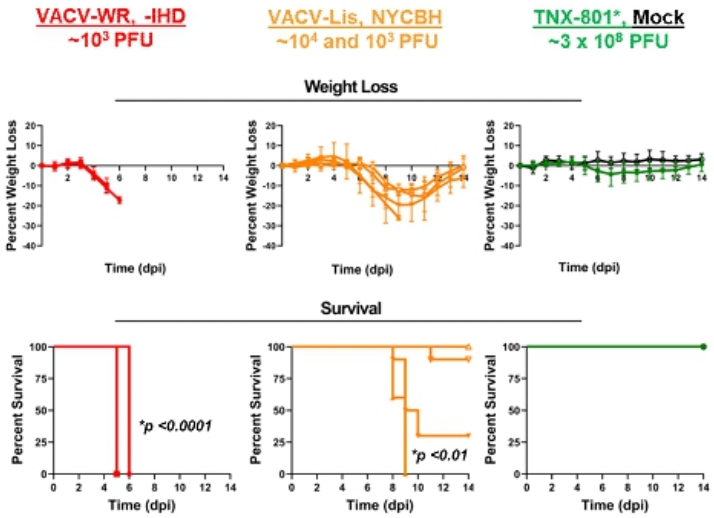
*100-1,000 higher infectious dose than VACV comparators

IND strain was deposited by the US Army in 1963

Farooq Nasar et al, Tonix unpublished data

TNX-801 Lacks Lethality Associated With Older Smallpox Vaccine Strains (Lister, NYC Board of health) in Double KO IFN-αR$^{-/-}$ and IFN-γR$^{-/-}$ Mice

(2 of 2)

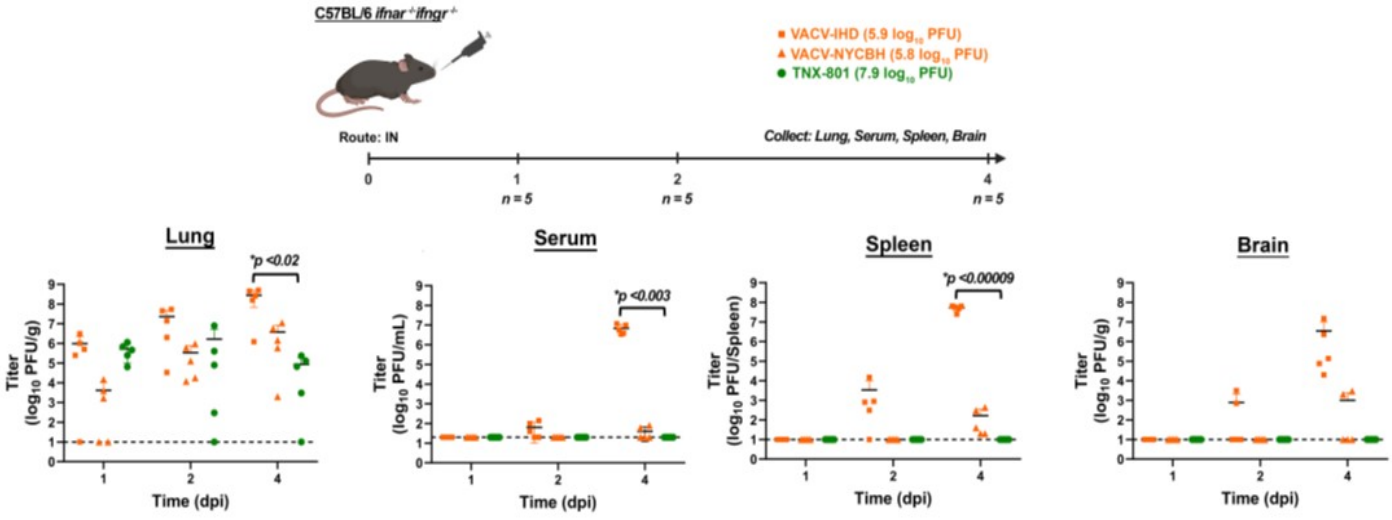


*10,000-100,000 higher infectious dose than VACV comparators

IND strain was deposited by the US Army in 1963

Farooq Nasar et al, Tonix unpublished data

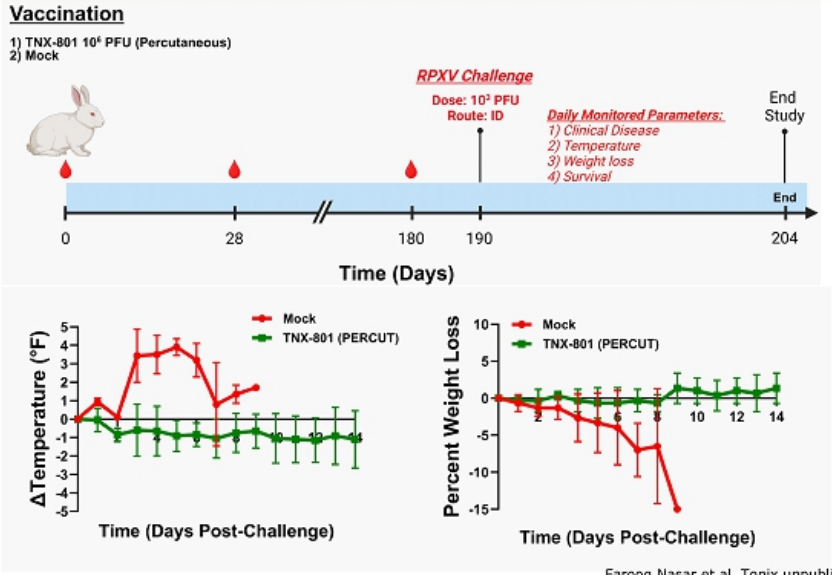
High Dose TNX-801 is Unable to Cause Disseminated Infection in Double KO IFN-αR$^{-/-}$ and IFN-γR$^{-/-}$ Mice



IND strain was deposited by the US Army in 1963

Single Vaccination of TNX-801 Provides Durable Protection Against Rabbitpox Virus (RPXV) Challenge

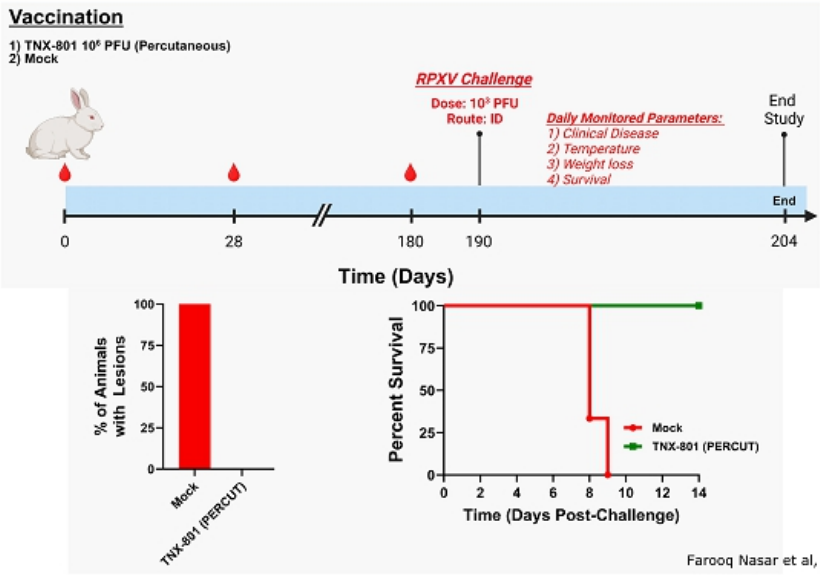
(1 of 2)



Farooq Nasar et al, Tonix unpublished data

Single Vaccination of TNX-801 Provides Durable Protection Against Rabbitpox Virus (RPXV) Challenge

(2 of 2)



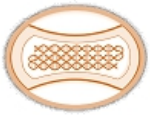
Farooq Nasar et al, Tonix unpublished data

Modern Cowpox and Vaccinia Are Endemic in the Environment Worldwide

Modern cowpox and vaccinia are ***environmentally endemic*** and are able to infect many animal hosts

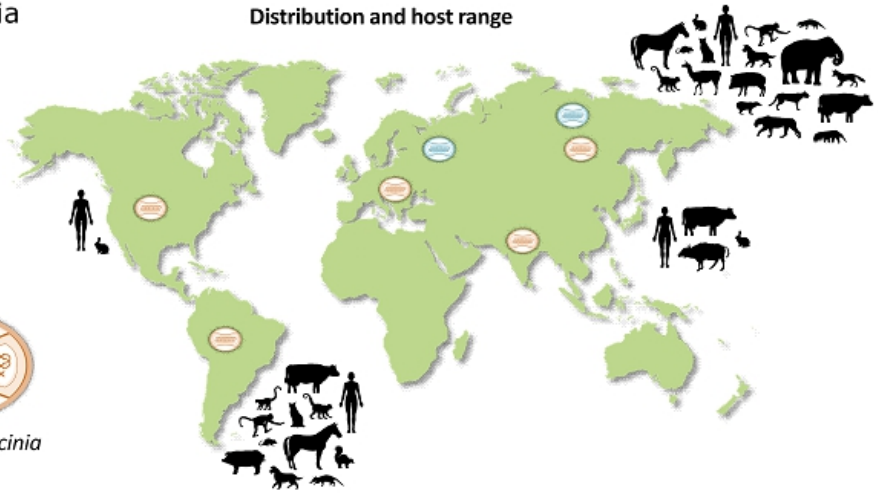
They are found widely distributed in important agricultural species and companion animals

Modern Cowpox



Modern Vaccinia

Distribution and host range



Silva NIO, et al. Viruses. 2020;13(1):43.

Commercial Applications of Licensed Recombinant Poxvirus-Based Vaccines

Agriculture[1,2]

- Newcastle disease virus (NDV) TROVAC (**recombinant fowlpox**)
- Avian influenza (AIV) TROVAC (**recombinant fowlpox**) (H5N9 and H5N1)

Cats[3]

- **Recombinant canarypox** rabies vaccine (ALVAC-RG) and feline leukemia (ALVAC-FeLV)

Dogs[4]

- RECOMBITEK® C4 **recombinant canarypox** vector expressing the HA and F glycoproteins of canine distemper virus; modified live adenovirus type 2, parainfluenza virus, and parvovirus

[1]Taylor J, et al. *Avian Dis.* 1996;40(1):173-180.
[2]The Poultry Site. March 14, 2005. Accessed July 9, 2021. https://www.thepoultrysite.com/news/2005/03/merial-launches-new-h5n1-avian-influenza-vaccine-provides-new-hope-for-avian-flu-epidemic
[3]Boehringer Ingelheim. Accessed July 9, 2021. https://www.boehringer-ingelheim.com/animal-health/companion-animals-products/purevax
[4]Larson LJ, et al. *Vet Ther.* 2007;8(2):101-106.

Approved Recombinant Poxvirus-Based Commercial Products[1-3]

Product	Application / disease	Location	Poxvirus vector	Host restricted?	Doses released to environment
TROVAC-AIV H5N1 *Boehringer Ingelheim*	Agriculture/avian influenza	Mexico, Central America	TROVAC-AIV H5N1	**No** ***Replication competent***	**2 billion** (as of 2006)
Purevax FeLV *Boehringer Ingelheim*	Companion animals (cats)/FeLV	US, others	ALVAC-FeLV Gag/Pol	Yes *Replication incompetent*	Unknown
Purevax Rabies *Boehringer Ingelheim*	Companion animals (cats)/rabies	US, others	ALVAC-RG	Yes *Replication incompetent*	Unknown
Recombitek *Boehringer Ingelheim*	Companion animals (dogs)/canine distemper	US, others	ALVAC-HA, F	Yes *Replication incompetent*	Unknown
Raboral V-RG **Rabisin** *Boehringer Ingelheim*	Wildlife control of rabies	US, Europe, Israel	Vaccinia Copenhagen RG	**No** ***Replication competent***	**250 million doses** *5 million doses/year*

[1]Boehringer Ingelheim. Accessed July 15, 2021. https://www.boehringer-ingelheim.com/animal-health/products
[2]Bublot M, Pritchard N, Swayne DE, et al. Development and use of fowlpox vectored vaccines for avian influenza. *Ann N Y Acad Sci.* 2006;1081:193-201.
[3]Maki J, Guiot AL, Aubert M, et al. Oral vaccination of wildlife using a vaccinia-rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG®): a global review. *Vet Res.* 2017;48(1):57.

Environmental Distribution: Vaccinia Released Aerially as Rabies Vaccine

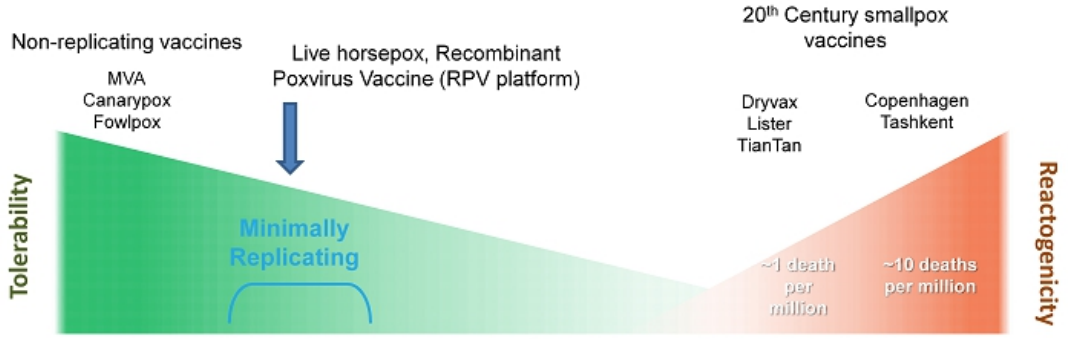
RABORAL V-RG® is an oral vaccine based on vaccinia–rabies-glycoprotein recombinant virus used to prevent the spread of rabies among wildlife populations[1-3,*]

- −RABORAL has been in continuous use since 1987
- −Approximately **250 million doses** in the form of animal baits have been aerially distributed across Europe, Israel, Canada, and the US at a rate of about 5 million baits per year
- −Species targeted include skunks, racoons, foxes, and coyotes
- −Jordona Kirby, the rabies field coordinator for the USDA's National Rabies Management Program was interviewed about dropping Raboral out of low-flying planes and helicopters to control rabies in the East Coast of the US[4]

*A registered trademark of Boehringer Ingelheim Animal Health

[1]Raboral V-RG®. Accessed July 9, 2021. https://www.raboral.com/about-rabies/raboral-v-rg
[2]Kieny MP, et al. *Nature*. 1984;312(5990):163-166.
[3]Maki J, et al. *Vet Res*. 2017;48(1):57.
[4]Science Friday : NPR Sept 30, 2022 NPR's program "Science Friday" at 30:02 in the podcast www.npr.org/podcasts/583350334/science-friday

Illustrative Safety Spectrum Of Pox-based Vaccine Vectors Optimizing Live Virus Vaccines



Replicative Capacity	Non-replicating	Minimally-replicating			Robustly replicating
#-of doses	Two	Single-dose			Single-dose
Durability of protection	waning	long			decades
Transgene expression	Poor	robust			robust

Horsepox Protection and Tolerability in Animals Potentially Decouples Protective Immunity from Reactogenicity

Conventional view holds that reactogenicity correlates with protection

Protective immunity is not necessarily related to reactogenicity

- –Reactogenicity was a basis for testing vaccine activity prior to the understanding that vaccinia was a virus

"Real World Evidence" supports efficacy of horsepox-like vaccines

- –Effectiveness of archaic vaccines (from the 1800's) support the belief that horsepox will be protective against smallpox
- –Historical evidence that horsepox-like vaccines prevented forward transmission

[1]Schrick, L. et al An Early American Smallpox Vaccine Based on Horsepox *N Engl J Med* 2017; 377:1491
[2]Tulman ER, et al. Genome of horsepox virus. *J Virol*; 2006 80(18):9244-58.PMID:16940536
[3]Brinkmann A et al, *Genome Biol*ogy 2020; 21:286 https://doi.org/10.1186/s13059-020-02202-0

Horsepox: More (Regulatory) Genes Confer Tolerability

For "20th Century vaccinia vaccines", the process of "Passage" through cows or birds was a primitive form of genetic engineering

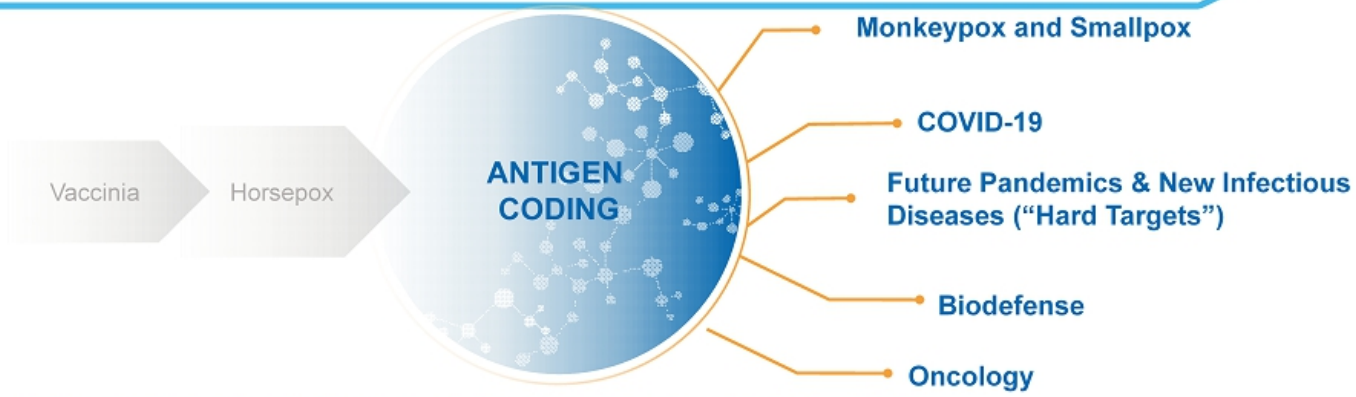
- –"Passage" through cows resulted in gene deletions that may have increased virulence relative to "circa 1860 vaccinia" (circa 1960 "vaccinia" deleted regulatory genes)
- –MVA: "Passage through birds resulted in extensive gene deletions that decreased replication in humans ("non-replicating")

Horsepox data: More Genes may be better than Fewer Genes

- –Horsepox appears to have preserved regulatory genes that confer tolerability, while preserving immune protection

[1]Schrick, L. et al An Early American Smallpox Vaccine Based on Horsepox *N Engl J Med* 2017; 377:1491
[2]Tulman ER, et al. Genome of horsepox virus. *J Virol*; 2006 80(18):9244-58.PMID:16940536
[3]Brinkmann A et al, *Genome Biol*ogy 2020; 21:286 https://doi.org/10.1186/s13059-020-02202-0

Live Virus Vaccine Platform: Recombinant Pox Vaccine (RPV) Platform



RPV VECTOR BELIEVED SIMILAR TO EDWARD JENNER'S VACCINE[1-3]

Using Proven Science To Address Challenging Disease States, We Have Created A Programmable Technology Platform Aimed At Combating Future Threats To Public Health

[1]Shrick, L. *N Engl J Med* 2017; 377:1491-1492. DOI: 10.1056/NEJMc1707600
[2]Esparza, *J. Vaccine*. 2020 Jun 19; 38(30): 4773–4779. doi: 10.1016/j.vaccine.2020.05.037
[3]Brinkmann, A. *Genome Biol*. 2020; 21: 286. doi: 10.1186/s13059-020-02202-0

TNX-1800 (SARS-CoV-2 spike – Expressing HPXV) Immunogenicity in Hamsters and Rabbits - 2023

viruses

MDPI

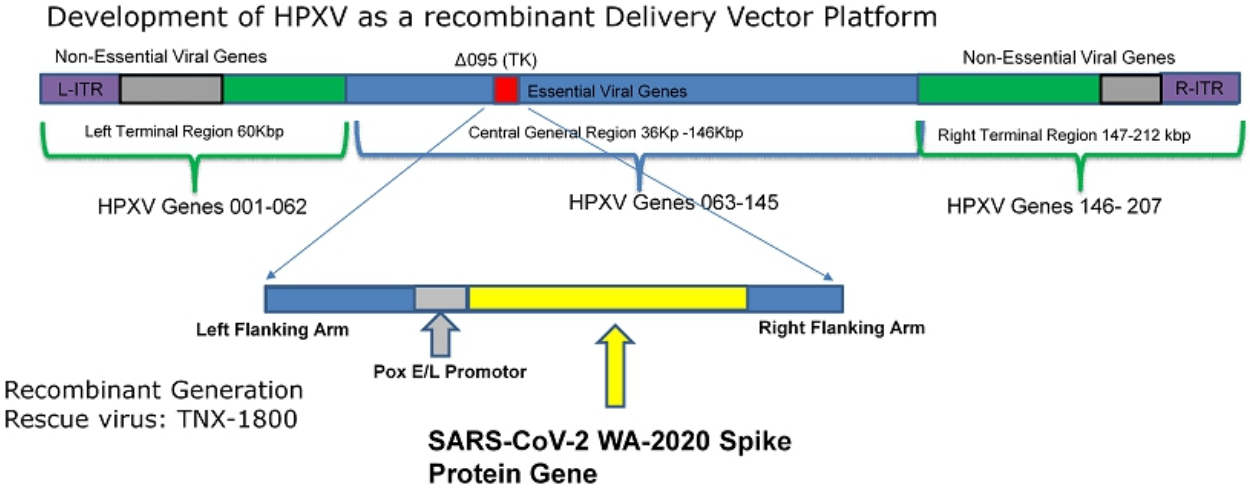
Brief Report

Immunogenicity and Tolerability of a SARS-CoV-2 TNX-1800, a Live Recombinant Poxvirus Vaccine Candidate, in Syrian Hamsters and New Zealand White Rabbits

Mayanka Awasthi [1], Anthony Macaluso [1], Scott J. Goebel [1], Erin Luea [2], Ryan S. Noyce [3], Farooq Nasar [1], Bruce Daugherty [4], Sina Bavari [1] and Seth Lederman [5,*]

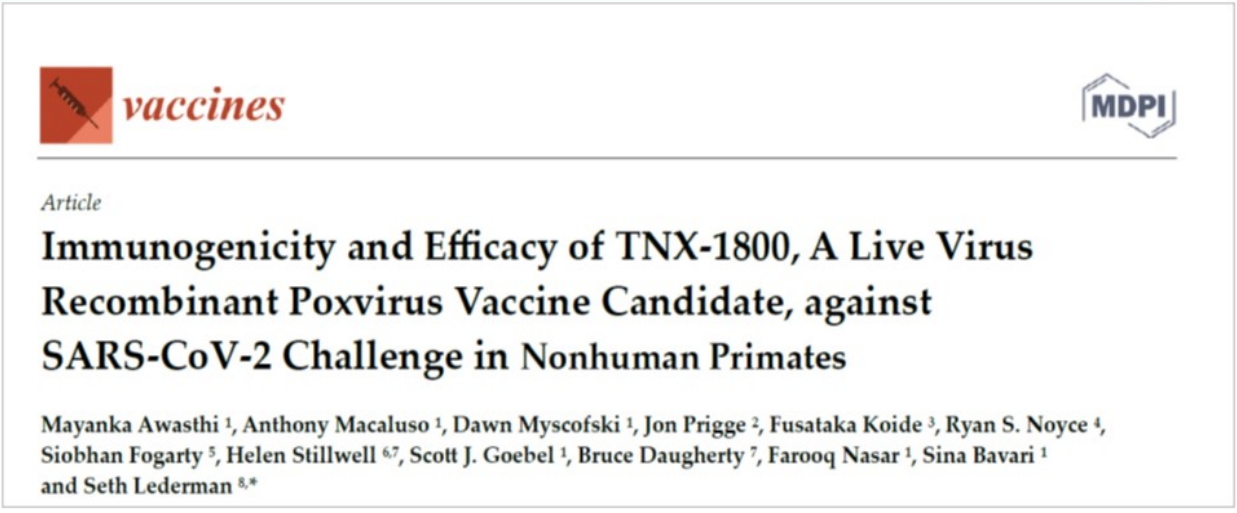
Awasthi M, et al. *Viruses*. 2023 Oct 21;15(10):2131. doi: 10.3390/v15102131. PMID: 37896908; PMCID: PMC10612059.

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



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

TNX-1800 Immunogenicity and Efficacy in NHPs - 2023



vaccines

MDPI

Article

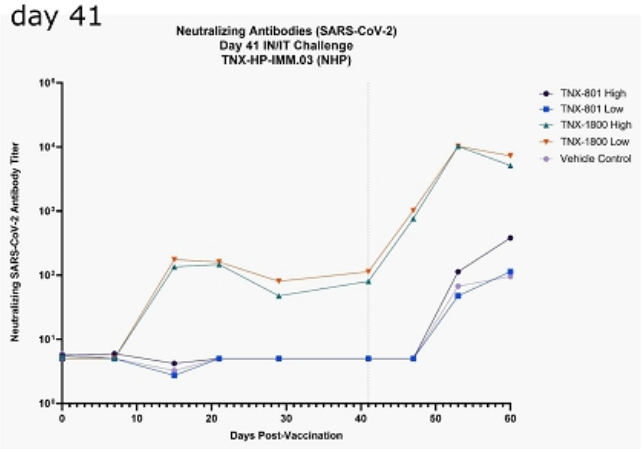
Immunogenicity and Efficacy of TNX-1800, A Live Virus Recombinant Poxvirus Vaccine Candidate, against SARS-CoV-2 Challenge in Nonhuman Primates

Mayanka Awasthi [1], Anthony Macaluso [1], Dawn Myscofski [1], Jon Prigge [2], Fusataka Koide [3], Ryan S. Noyce [4], Siobhan Fogarty [5], Helen Stillwell [6,7], Scott J. Goebel [1], Bruce Daugherty [7], Farooq Nasar [1], Sina Bavari [1] and Seth Lederman [8,*]

Awasthi M, et al. *Viruses*. 2023 Oct 21;15(10):2131. doi: 10.3390/v15102131. PMID: 37896908; PMCID: PMC10612059.

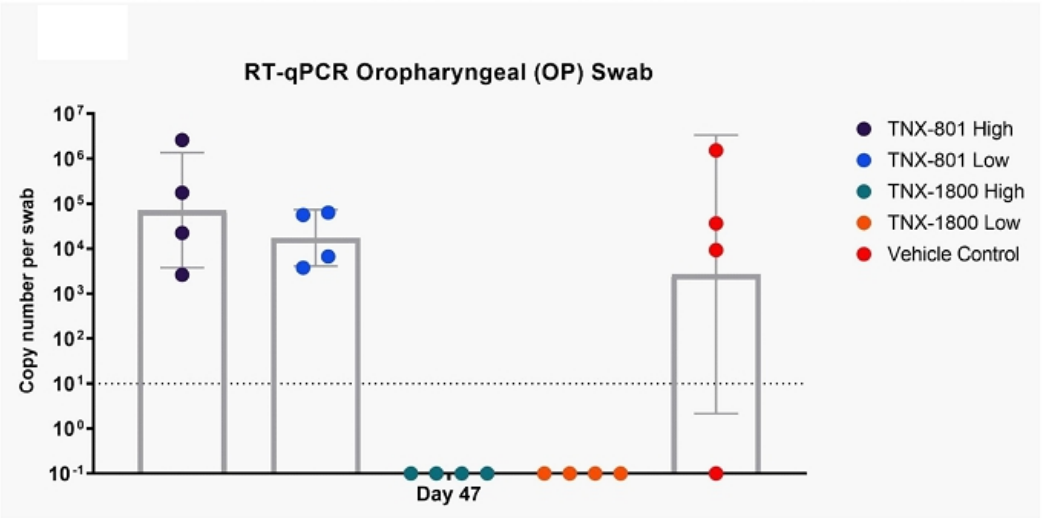
Immunogenicity: All NHPs in TNX-1800 Vaccinated Group Had Neutralizing Antibody Response

NHPs were vaccinated day 0 and challenged day 41



Vaccination with TNX-1800 results in the inhibition of SARS-CoV-2 Replication in Vaccinated NHPs

NHPs were vaccinated day 0 and challenged day 41; "Day 47" is 6 days after challenge



TNX-801 is Potential Vaccine for Mpox and Smallpox
Platform to express other viral antigens

Animal studies show TNX-801 protects against mpox
- –Appears to provide mucosal immunity after percutaneous vaccination (May prevent forward transmission)

Single dose efficacy
- –May elicit durable or long-term protection by stimulating T cell ("cell-mediated") immunity

Potential to manufacture at scale
- –Low dose because replication amplifies dose *in vivo*

Standard cold chain believed to be sufficient for shipping and storage

Jenner's vaccinia is the oldest vaccine technology – can now be engineered with payload antigens
- –"Jenner's vaccinia" and its descendants "circa 1960 Vaccinia" eradicated smallpox
- –"20th Century vaccinia" kept mpox out of the human population in Africa
- –Horsepox and vaccinia express transgenes with high fidelity

Tonix Platform Selected by NIH/NIAID : Project NextGen COVID


Nasdaq Market Activity News + Insights Solutions About Nasdaq+

Tonix Pharmaceuticals' Vaccine Candidate, TNX-1800, Selected by NIH/NIAID Project NextGen for Inclusion in Clinical Trials

PUBLISHED
NOV 2, 2023 8:00AM EDT

Feedback

NIAID is conducting early phase clinical trials on select next generation COVID-19 vaccine candidates with the intent to identify promising vaccine candidates

TNX-1800, a live virus percutaneous vaccine candidate, is based on Tonix's recombinant pox virus (RPV) platform

Phase 1 clinical trial of TNX-1800 expected to start in the second half of 2024

NIAID will cover the full cost of the clinical trial; Tonix will supply the vaccine candidate

Tonix has Capacity and Technology to Develop and Produce TNX-801 and Other HPXV Vaccines

Potential to manufacture at scale

–Low dose because replication amplifies dose *in vivo*

Believed will be thermo-stable in ultimate lyophilized formulation

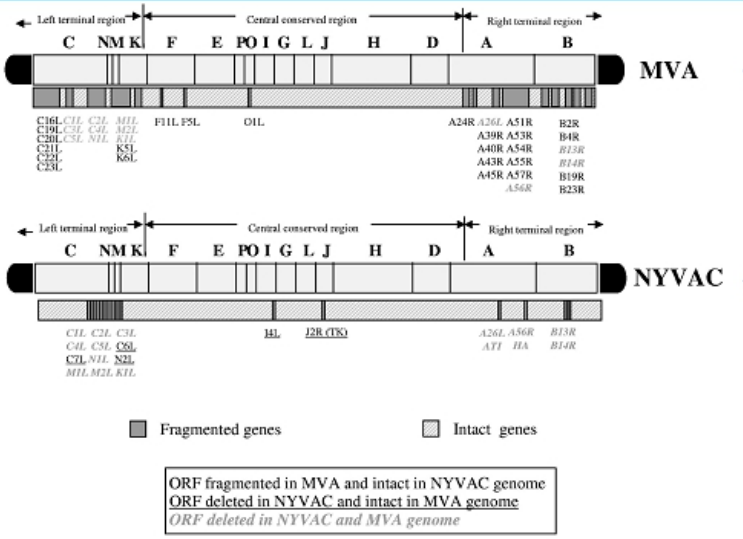
R&D Center- Maryland
Operational BSL-3 capable

Advanced Manufacturing Center- MA
GMP-manufacturing capability*

*GMP Suites currently decommissioned

"Classical" Technologies for Attenuating Pox Vaccines

Deletions by Serial Passage vs Targeted Genomic Modification[1]

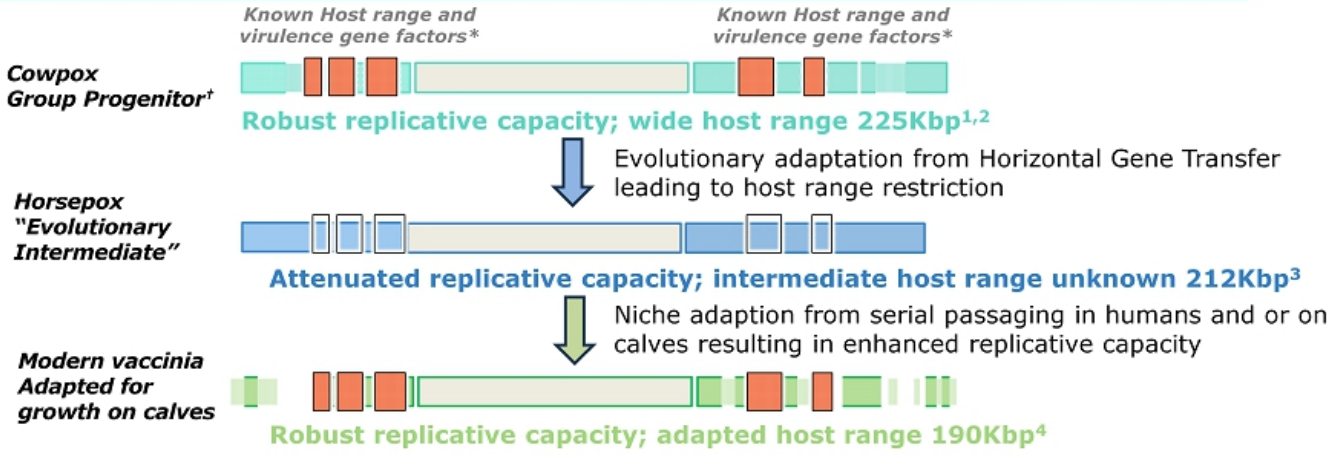


Serial Passage (1970's)
In primary chick embryo fibroblast (CEF's) resulting in numerous attenuating genomic deletions (*e.g.,* MVA)

Targeted genomic manipulation (1990's)
Systematic deletion of known virulence and host range factors (*e.g.,* Enzo Paoletti[2] and Virogenetics)

[1]Nájera JL, et al. *J Virol.* 2006 Jun;80(12):6033-47. doi: 10.1128/JVI.02108-05. PMID: 16731942; PMCID: PMC1472566.
[2]https://en.wikipedia.org/wiki/Enzo_Paoletti

Interpretation of Evolution and Real-World Evidence to Recreate an Extinct Virus Vaccine: Larger "Model"



*This is a conceptual view to illustrate whether these genes are active or not and does not indicate the actual number, size, or location of the genes
†Stripes indicate regions among different vaccinia strains that are present in some but absent in others

1. Tulman ER, et al. *J Virol.* 2006;80(18):9244-9258.
2. Schrick L, et al. *N Engl J Med.* 2017;377(15):1491-1492.
3. Dabrowski PW, et al. *PLoS One.* 2013;8(12):e79953.
4. Tulman ER, et al. *J Virol.* 2006;80(18):9244-9258.
5. Qin L, et al. *J Virol.* 2015;89(3):1809-1824.

Recreating an Extinct Vaccine Virus

"Evolutionary Intelligence": Unknown evolutionary pressures resulted in Horsepox

- –Harnessing the results of large numbers of genetic events: both deletions and reactivations
  - In the parlance of "AI/Large Language Models" – A very large "model"
  - A better term may be: "*Evolutionary Design*"?
- –Not limited by knowledge of:
  - Functions of many viral genes
  - Functional interactions/interplay of viral genes

Real World Evidence for >200 years includes:

- –Activity in preventing smallpox
- –Tolerability in humans
- –Control of human and animal transmission by hand-washing and modern animal husbandry

Selection: Jenner played an ACTIVE role in identifying "cowpox"/"horsepox" as a safer vaccine

- –Jenner was not just "lucky" – he tried more than once
- –Jenner was one of several contemporaries who were searching among variola and animal vaccines for improved variolation technology
- –His "laboratory" was a community with periodic outbreaks of smallpox, cowpox and horsepox

Contributors

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[2]IITRI
[3]National Toxicology Program (NTP) at National Institute of Environmental Health Sciences (NIEHS), NIH; Artic Slope Regional Corp.

Selected Publications

1. Noyce RS, Lederman S, Evans DH. Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. PLoS One. 2018 Jan 19;13(1):e0188453. doi: 10.1371/journal.pone.0188453. PMID: 29351298; PMCID: PMC5774680.

2. Esparza J, Lederman S, Nitsche A, Damaso CR. Early smallpox vaccine manufacturing in the United States: Introduction of the "animal vaccine" in 1870, establishment of "vaccine farms", and the beginnings of the vaccine industry. Vaccine. 2020 Jun 19;38(30):4773-4779. doi: 10.1016/j.vaccine.2020.05.037. Epub 2020 May 27. PMID: 32473878; PMCID: PMC7294234.

3. Noyce RS, Westfall LW, Fogarty S, Gilbert K, Mpanju O, Stillwell H, Esparza J, Daugherty B, Koide F, Evans DH, Lederman S. Single Dose of Recombinant Chimeric Horsepox Virus (TNX-801) Vaccination Protects Macaques from Lethal Monkeypox Challenge. Viruses. 2023 Jan 26;15(2):356. doi: 10.3390/v15020356. PMID: 36851570; PMCID: PMC9965234.

4. Awasthi M, Macaluso A, Myscofski D, Prigge J, Koide F, Noyce RS, Fogarty S, Stillwell H, Goebel SJ, Daugherty B, Nasar F, Bavari S, Lederman S. Immunogenicity and Efficacy of TNX-1800, A Live Virus Recombinant Poxvirus Vaccine Candidate, against SARS-CoV-2 Challenge in Nonhuman Primates. Vaccines (Basel). 2023 Nov 2;11(11):1682. doi: 10.3390/vaccines11111682. PMID: 38006014; PMCID: PMC10674175.

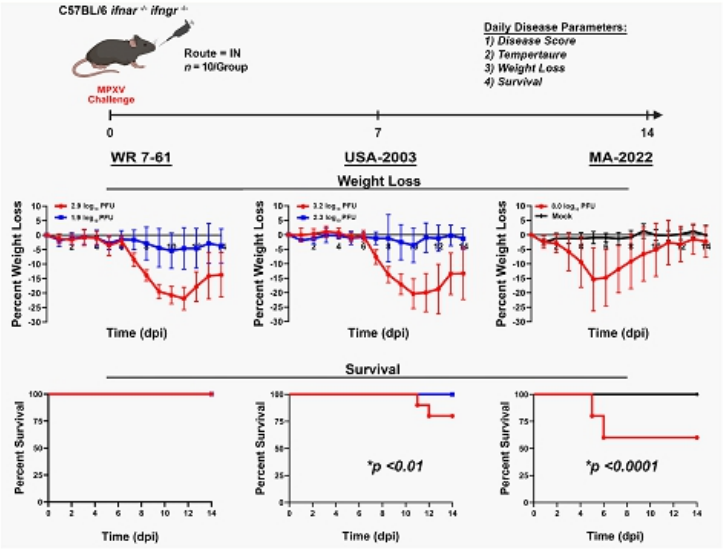
5. Awasthi M, Macaluso A, Goebel SJ, Luea E, Noyce RS, Nasar F, Daugherty B, Bavari S, Lederman S. Immunogenicity and Tolerability of a SARS-CoV-2 TNX-1800, a Live Recombinant Poxvirus Vaccine Candidate, in Syrian Hamsters and New Zealand White Rabbits. Viruses. 2023 Oct 21;15(10):2131. doi: 10.3390/v15102131. PMID: 37896908; PMCID: PMC10612059.

6. Trefry SV, Awasthi M, Raney CN, Cregger AL, Gonzales CA, Layton BL, Enamorado RN, Martinez NA, Gohegan DS, Masoud-Bahnamiri M, Cho JY, Myscofski DM, Moulaei T, Ziółkowska NE, Goebel SJ, Lederman S, Bavari S, Nasar F. Recombinant chimeric horsepox virus (TNX-801) is attenuated relative to vaccinia virus strains in both *in vitro* and *in vivo* models. mSphere. 2024 Dec 19;9(12):e0026524. doi: 10.1128/msphere.00265-24. Epub 2024 Nov 13. PMID: 39535212; PMCID: PMC11656774.

Thank You

MPXV clade IIb (MA-2022) is 10,000- to 100,000-fold more attenuated than clade IIa (WR 7-61 and US-2003)

Double KO IFN-αR$^{-/-}$ and IFN-γR$^{-/-}$ mice



Farooq Nasar et al, Tonix unpublished data