National Center for Emerging and Zoonotic Infectious Diseases

Report on work with live variola virus at the Centers for Disease Control and Prevention

Presented by Christina Hutson, Ph.D., M.S.
Chief, Poxvirus and Rabies Branch (PRB)

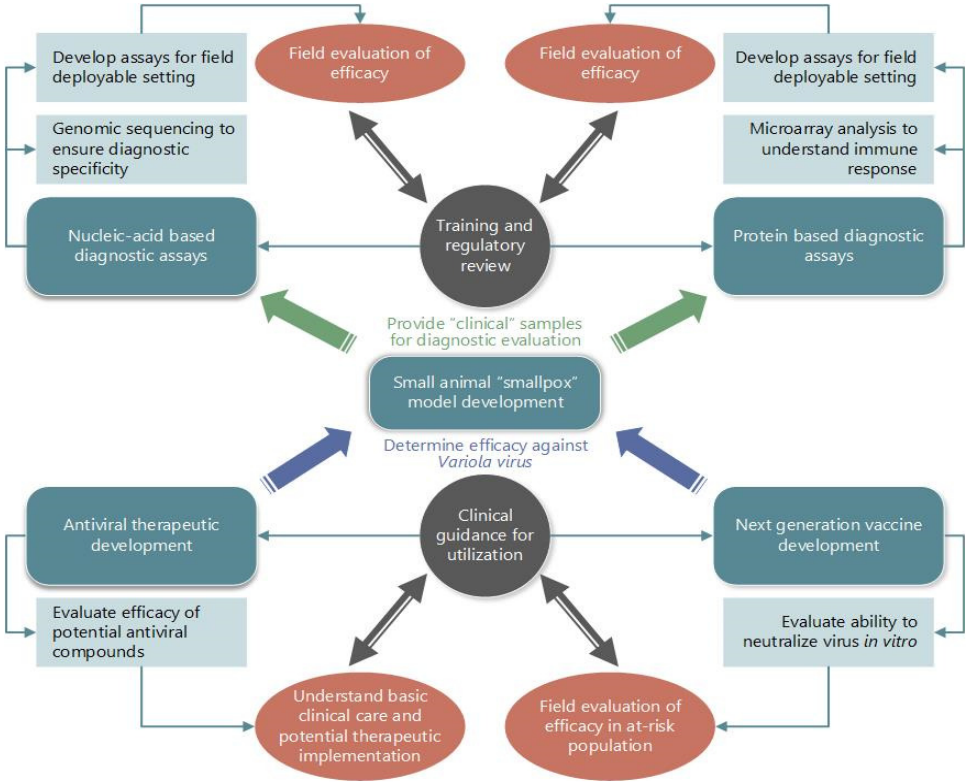
PRB, DHCPP, NCEZID, CDC

World Health Organization Collaborating Center for Smallpox and other Poxvirus Infections

United States Smallpox Research Agenda

- Two laboratories in the world WHO sanctioned to work with live variola virus
  - US CDC and Russia-VECTOR

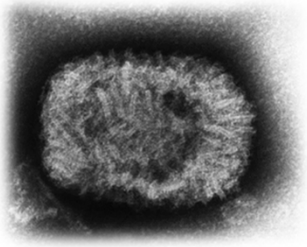
- The US Smallpox Research Agenda was initiated based on the expert review "Assessment of Future Uses for Live *Variola virus*" by the Institute of Medicine in 1999[1]

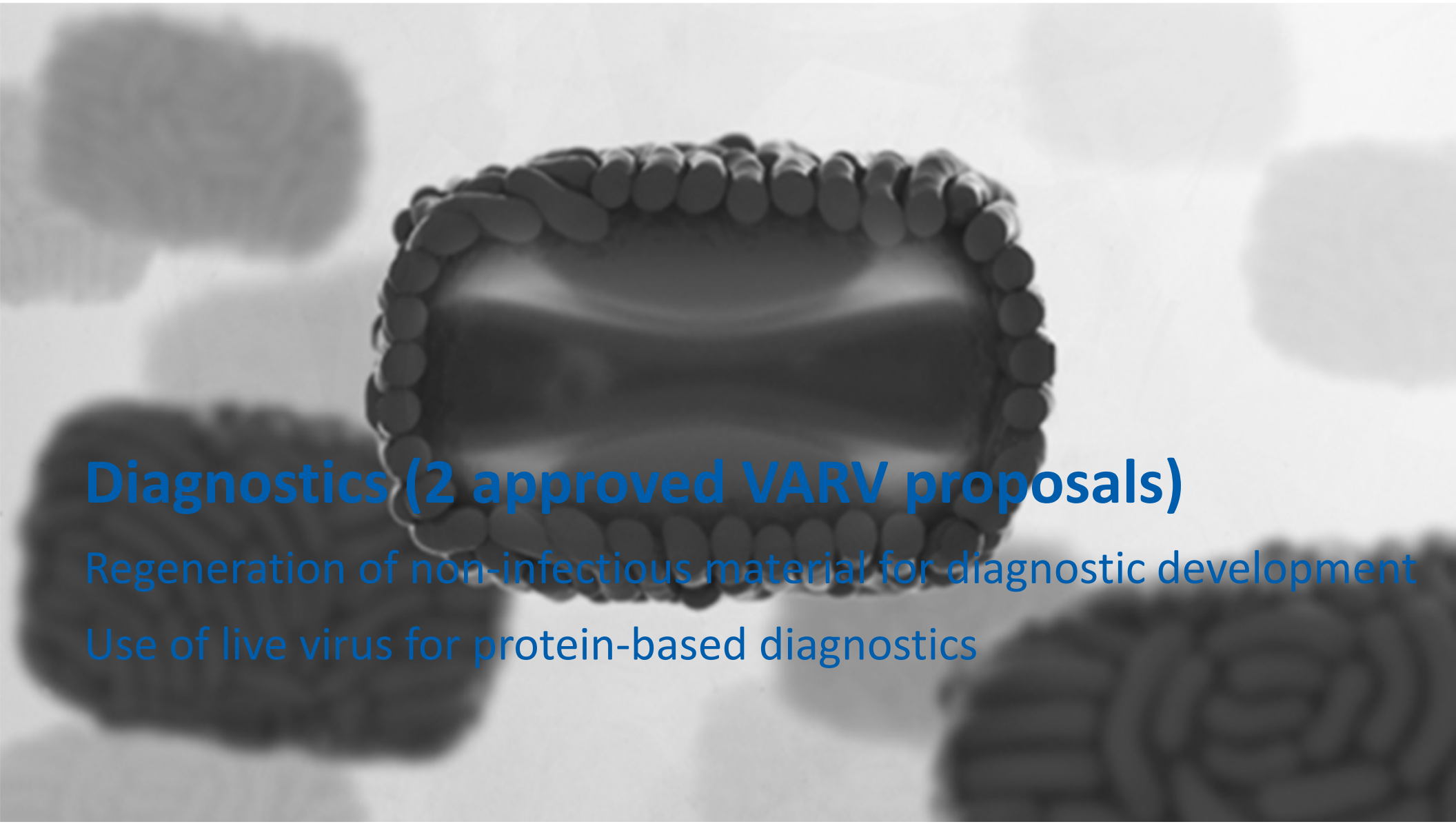


http://www.nationalacademies.org/hmd/Reports/1999/Assessment-of-Future-Scientific-Needs-for-Live-Variola-Virus.aspx

CDC Proposals to Work with Live VARV

- WHO Advisory Committee to Variola Virus Research (ACVVR) votes annually on all work with live VARV
  - Previous compelling results with other OPXV surrogates
    - Including *in vivo* OPXV work if applicable
    - Need for work with live VARV
    - FDA requests (e.g., post-marketing commitment (PMCs))
- Currently we have 8 proposals approved to work with live agent
  - Diagnostics (2)
  - Vaccines (2)
  - Therapeutics (3)
    - Mouse model (1)



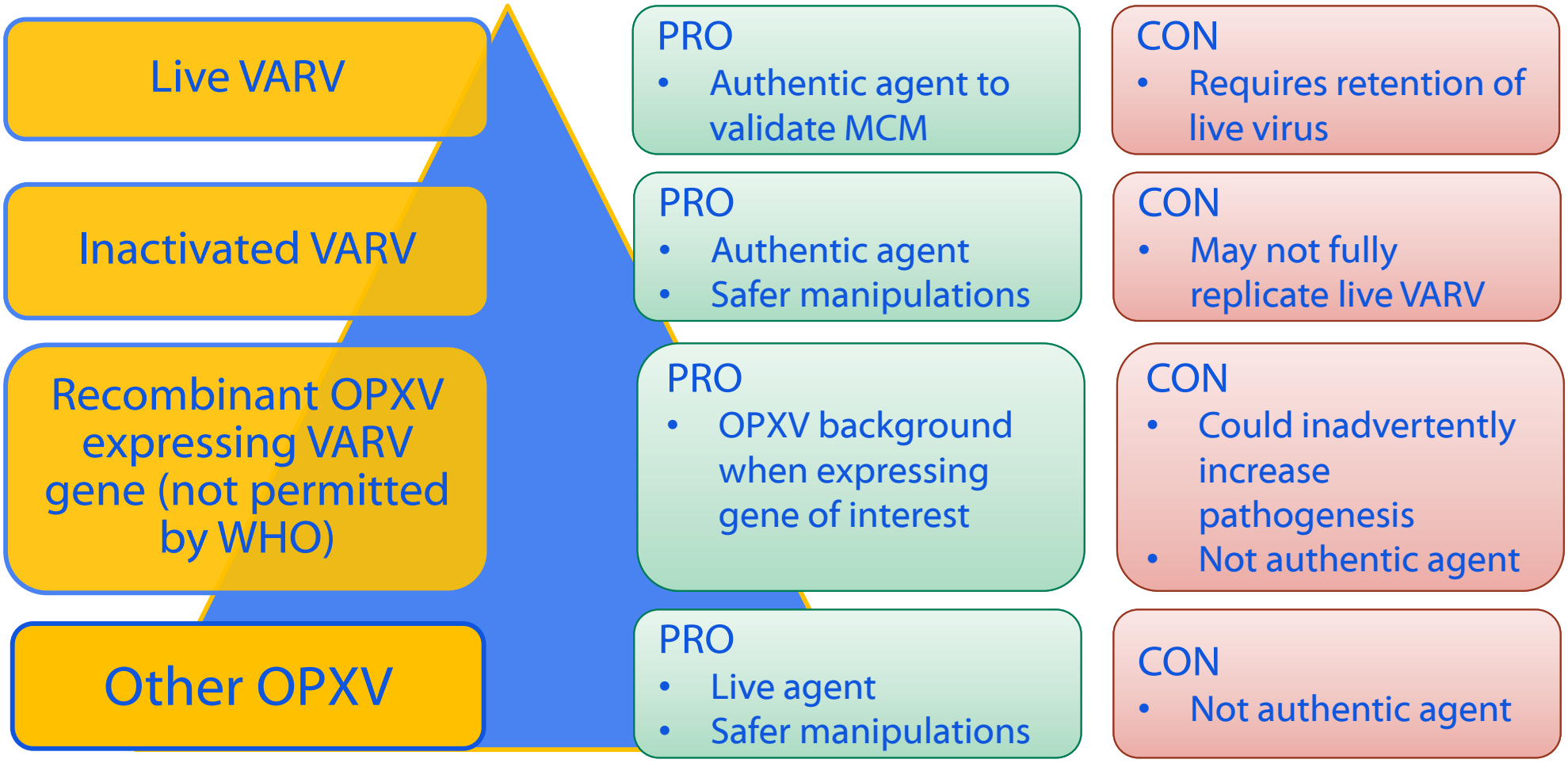


Diagnostics (2 approved VARV proposals)

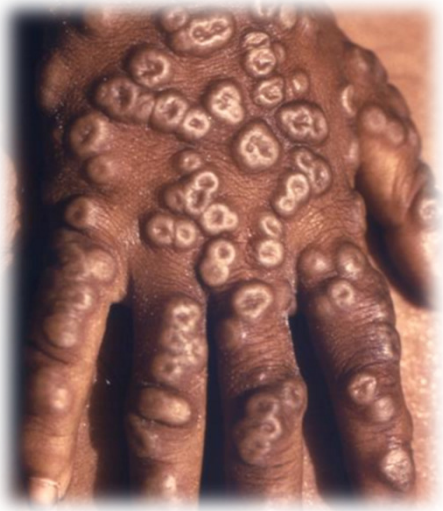
Regeneration of non-infectious material for diagnostic development

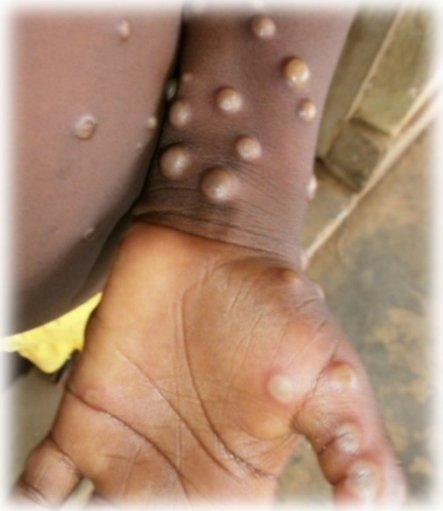
Use of live virus for protein-based diagnostics

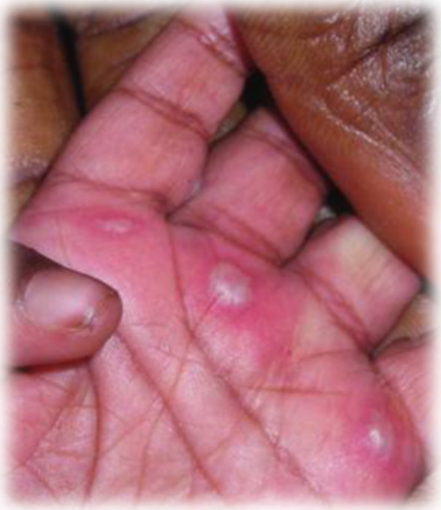
Use of Live VARV for Diagnostic Assay Development



Public health need or potential benefit:
Necessary to have accurate tests available to differentiate rash illnesses







Current Smallpox Diagnostic Testing

- Laboratory Response Network (LRN)
  - Integrated network of laboratories
    - State and local public health
    - Federal (United States)
    - Military
    - International
  - Respond to bioterrorism and other public health emergencies
    - FDA cleared OPXV tests developed by the CDC
      - Non-variola orthopoxvirus test
      - Variola specific
      - Orthopoxvirus generic*
  - Ongoing clinical algorithm and testing collaboration/training

Laboratory Information | CDC

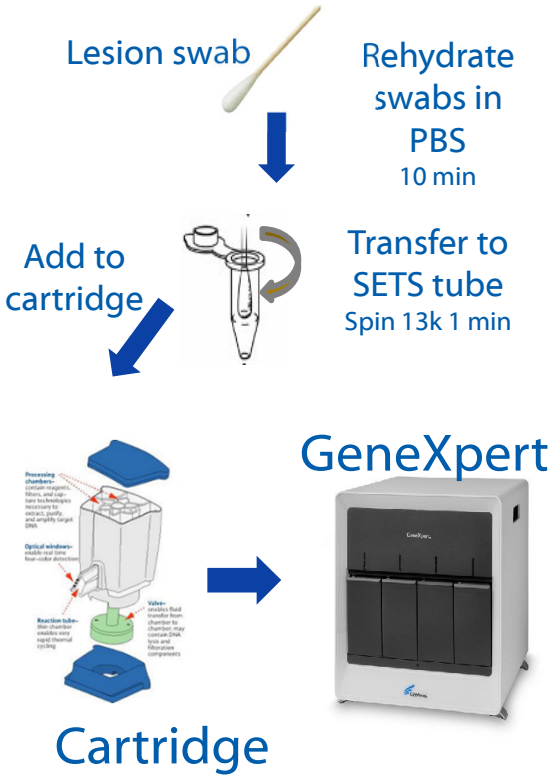
Diagnostic assessment with new VARV sequences

- Testing algorithm (e.g. NVO, VARV and OPX3) ensures confidence in results
  - New isolates (e.g. Alaskapox virus) can confound test results
  - Alaskapox virus results in NVO negative, OPX3 positive=looks like VARV
    - Cases in 2020, 2021 and 2023
  - Continual assessment of tests used within the LRN and at CDC is needed

- Tests assessed with newly sequenced VARV isolates *in silico* to determine if wet lab testing is needed

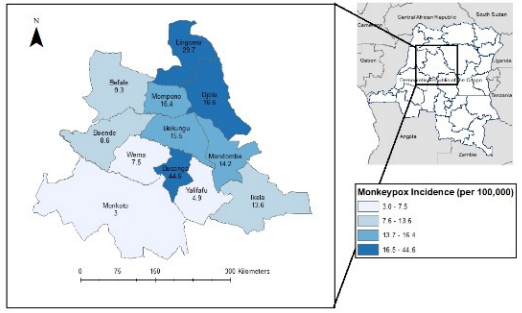
Previous work with GeneXpert

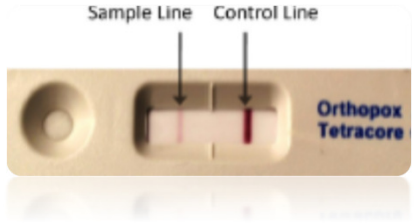
- National Reference Laboratory (Kinshasa, DRC) successfully detects Monkeypox with GeneXpert
  - **Li D, et al. Evaluation of the GeneXpert for Human Monkeypox Diagnosis. Am J Trop Med Hyg. 2017 Feb 8;96(2):405-410. doi: 10.4269/ajtmh.16-0567. Epub 2016 Dec 19. PMID: 27994107; PMCID: PMC5303045.**
  - Lesion specimens collected for diagnosis
  - Results compared to MPX-generic real-time PCR assay (gold standard)
- Multiplex VARV specific assays on GeneXpert
  - Detect VARV (10X LoD) in contrived clinical samples using multiplex test
  - Problems highlight the need for testing with authentic agent
    - DNA + reagents was positive
    - Whole virus + reagents was negative (problem with extraction)
    - Whole virus + reagents + buffer was positive



Point-of-care diagnostic use in resource limited area

- Evaluate *Orthopoxvirus* generic LFA commercially produced
  - Laboratory evaluation: Sensitive to 10^6 pfu/ml
  - In-field evaluation – Boende, Tshuapa, DRC (partners KSPH, INRB, MOH)
    - Protocol approved (CDC IRB and KSPH IRB)
    - 36 enrolled and tested
    - High specificity: low sensitivity
    - Dramatic decreases with time to confirmation
      - Disease onset to LFA : 4.5 days (range 0-14 days)
      - Disease onset to PCR : 30.3 days (range 7-72 days)
    - Efforts to analyze missing specimens and publish results are ongoing
    - High-sensitivity antigen detections assays – bead based

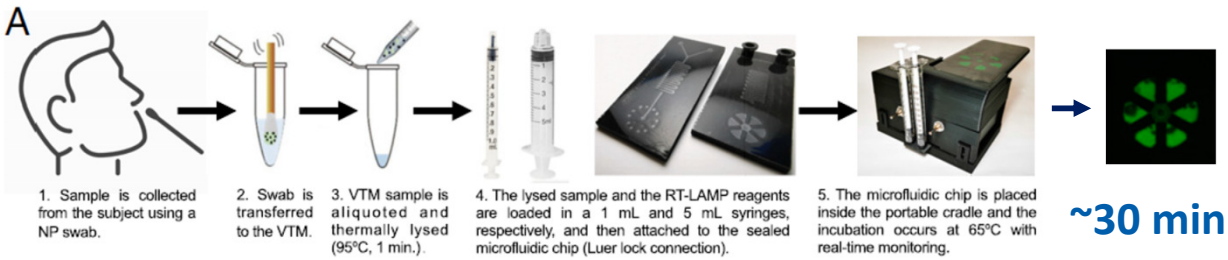




Townsend, et. al.. J Virol Methods. 2013 Jan;187(1):37-42.

Rapid isothermal amplification and portable detection system

- Advantages
  - Minimal manipulations
  - Rapid (30 min reaction time)
  - Detection via smartphone camera



SARS-CoV-2 LAMP detection

Specificity	USA2022	West-african	Congo basin	pan-OPXV	Variola	Sequence-specific Disruption
Clade II	X	X	-	-	-	Loop 2' structure disruption (F1c - 289bp gap)
pan-MPXV	X	X	X	-	-	Strand-displacement disruption (F3 - 27bp gap)
pan-OPXV	X	X	X	X	X	N/A

- Specificity expected with pox-specific LAMP assay
- Assay specificity & sensitivity with laboratory isolates
- Assay Sensitivity with clinical isolates ~93-98%
- Chip has 4 channels

Orthopoxvirus LAMP detection in collaboration with Arizona State University (ASU)

Ganguli A, et al. PNAS (2020)

Summary and project continuation goals -Diagnostics

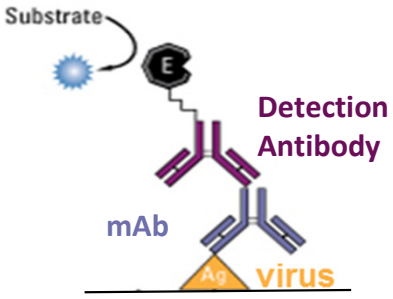
- Maintain VARV DNA and antigen stocks at the WHO Collaborating Centre (WHO CC)
  - Assay validation is substantially more robust when validated with extracted genomic DNA, rather than plasmids expressing the target portions of genomic DNA
  - ➢ Nucleic-acid diagnostic assays must be validated against new sequences/isolates and transitioned to newer platforms/reagents as technology advances (FDA submission)
    - New *Orthopoxviruses* continue to be identified
    - Expand instruments and commercial inventory
      - As previous instruments and/or reagents are discontinued
      - Mitigate against supply chain vulnerabilities and automate available PCR tests

Summary and project continuation goals (cont.)

- Multiplexed POC assays for detection of variola virus to an automated platform (GeneXpert) expands potential for surveillance
  - Implementation within two laboratories within DRC (OPXV/MPXV) with promising results
- Protein-based diagnostic tests are still being explored
  - LFA did not have sufficient sensitivity
  - New collaborations may result in further advancements
- LAMP diagnostic assays provide valuable flexibility and rapid testing for potential field deployment
  - Field deployable diagnostics will be critical should VARV (re)emergence occur
  - Initial results for LAMP assay are encouraging

Challenges for assay validation without using live Variola virus

			D8			H3			A25				A33			H3	
	Virus (pfu/mL)	Ctrl	1	20	22	11	13	15	40	47	49	50	29	37	Non-Pox	3B6	E2
Live VARV	10^7	0	0.84	-0.16	-0.04	0.06	-0.18	-0.14	0.76	1.76	1.7	0.12	-0.15	0.74	0	1.97	1.78
	10^6	0	0.19	-0.04	-0.01	-0.02	0.01	-0.03	0.16	1.25	1.61	0.06	-0.03	0.05	0	1.4	0.39
	10^5	0	0.02	-0.01	0.02	0	0.04	-0.01	0.01	0.19	0.45	0.07	0	0.02	0	0.28	-0.03
Gamma-Irradiated VARV	10^7	0	1.11	-0.13	0.35	1.21	0.87	-0.01	1.22	1.59	1.58	0.09	-0.09	0.57	0	1.73	1.8
	10^6	0	0.47	-0.01	0.09	0.46	0.15	-0.01	0.37	1.24	1.78	0.08	0.07	0.05	0	1.36	0.42
	10^5	0	0.05	0	0.04	0.04	0.06	0	0.04	0.23	0.55	0.08	0.01	0.03	0	0.24	-0.03



- Binding ELISA
  - Paired sample of live and γ-irradiated (inactivated) VARV
    - VARV coated wells
  - Values are background subtracted (from Ctrl/Non-Pox mAbs)
- Some mAbs differentially bind live vs. γ-irradiated virus
  - 22 (D8); 11 and 13 (H3) unable to capture live virus

Mpox outbreak lessons learned -Diagnostics

- First Mpox case tested at the Massachusetts LRN laboratory using FDA cleared NVO test developed for smallpox preparedness
  - 510k updates needed-automated extraction platforms, additional PCR platforms and reagents
- US LRN laboratories had capacity to test approximately 6,000-10,000 per week
  - To improve access/capacity, CDC worked with FDA, APHL and other partners to onboard the NVO test in 5 commercial labs in < 2 months
  - Commercial laboratory testing added ~70,000 additional tests/week
  - Testing capacity ramped up from 10K to 80K tests per week
- Developing countries/remote settings need point-of-care and/or field deployable tests

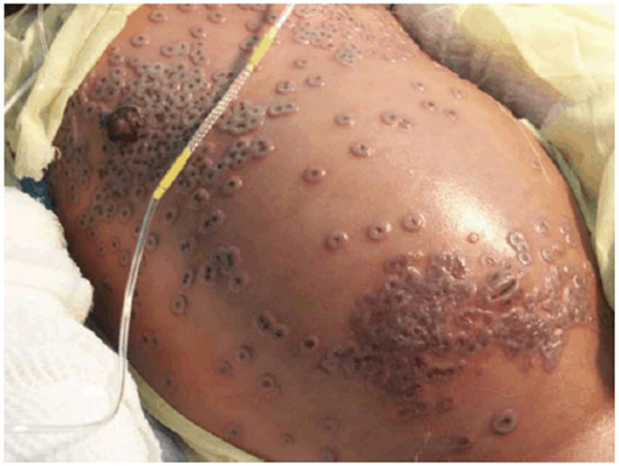
Vaccines (2 approved VARV proposals)

JYNNEOS & Lc16m8

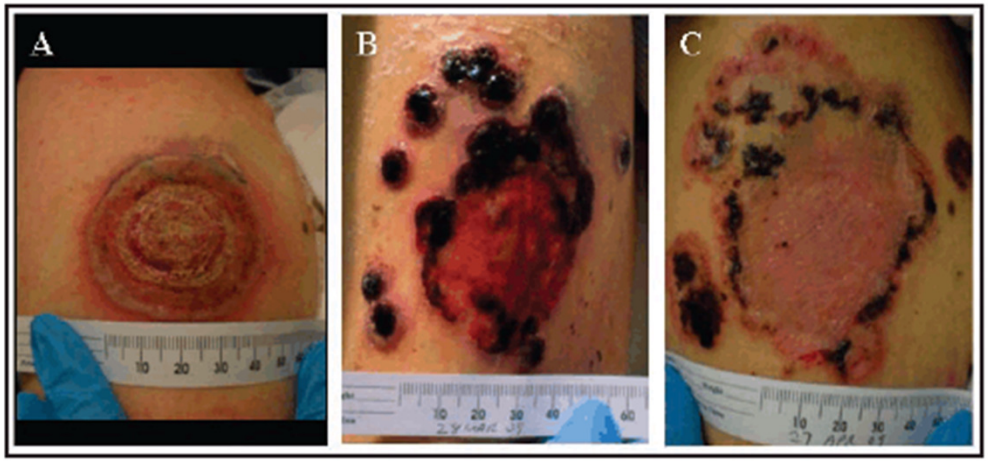
mRNA vaccine (new proposal approved in late 2023)

Traditional Smallpox Vaccine Complications

- Live Vaccinia virus (smallpox vaccine)
  - 1776, Edward Jenner
  - Used during global eradication campaign
- Potential severe complications:
  - Progressive vaccinia
  - Eczema vaccinatum
  - Postvaccinial encephalitis
  - Autoinoculation/inadvertent transmission
  - Ocular infections
  - Myo/pericarditis
  - Fetal vaccinia
  - Death (especially in infants/elderly)



Eczema vaccinatum



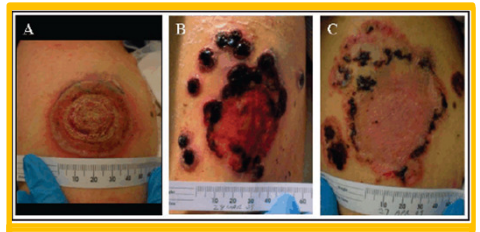
Progressive vaccinia

Smallpox Vaccine Advances

- Traditional (first generation, Dryvax®)
  - Propagated on calf skin
- Second generation (ACAM2000)
  - Propagated in tissue culture
  - Good manufacturing practices
- Third generation (JYNNEOS®, LC16m8)
  - Attenuated live virus
  - Propagated in tissue culture
  - Good manufacturing practices
- Stockpiled in US Strategic National Stockpile
  - JYNNEOS-Approved by US Food & Drug Administration (Sept 2019)
  - 1,280,144 doses Administered in the 57 U.S. Jurisdictions as of September 26, 2023

Use of *Variola virus* to support "novel" vaccine development

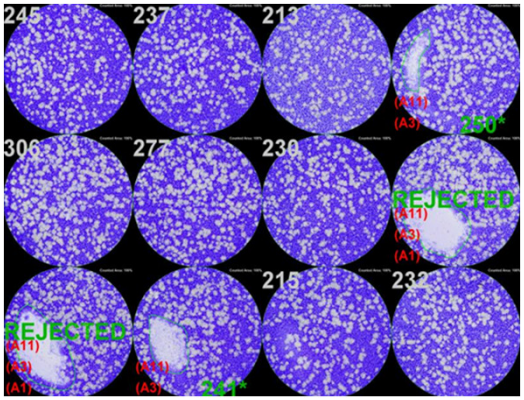
- Exploring next generation vaccine development
  - mRNA vaccine
- Continued focus on "third generation" vaccine development
  - Never tested directly against smallpox
- Bridging studies to existing vaccine immune response
  - Importance of vaccine elicited humoral response in protection
  - Critical for "third generation" vaccines which do not elicit a "take"
- Role of *Variola virus* PRNTs in evaluation of vaccines
  - Statistically significant difference has been observed between neutralization of different virus antigens
    - Modified Vaccinia Ankara (MVA) vs. Dryvax® vaccinations
    - LC16m8 vs. Dryvax ® vaccinations



Progressive vaccinia in a military patient post vaccination. Image from Lederman et al., 2009

JYNNEOS™ non-inferiority clinical study (part of licensure submission)

- JYNNEOS™-Collaboration with Bavarian Nordic
  - Phase II clinical trial with 200 vaccinia-naïve subjects
    - JYNNEOS™ versus ACAM2000™
      - Sera taken pre and peak time post vaccination regimen
    - Primary endpoint – VACV-WR neutralization
    - *Variola virus* – Bangladesh Solaiman: IMV neutralization as secondary endpoint
      - Subset to be tested for ability to neutralize *Variola virus at request of US Food and Drug Administration*
      - *Variola virus Plaque Reduction Neutralization Test (PRNT)*

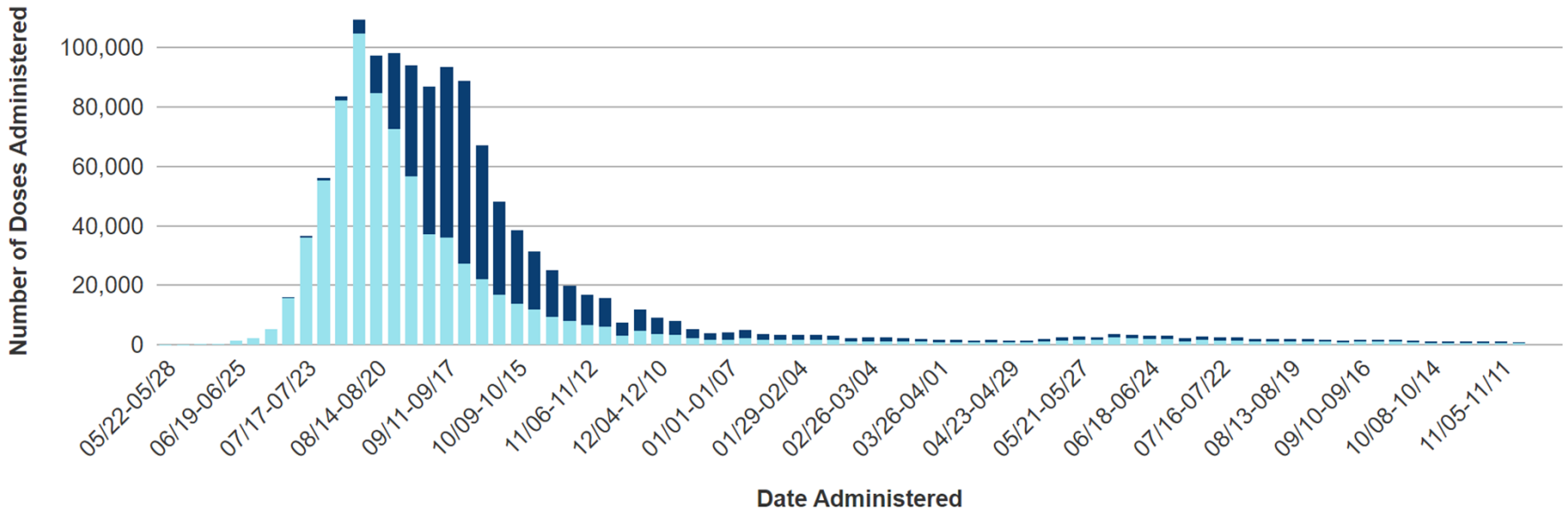


JYNNEOS Use during mpox outbreak

1,280,114

Doses Administered in the 57 U.S. Jurisdictions Reporting Data as of November 28 2023 .

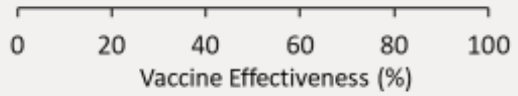
Total JYNNEOS Vaccine Second Doses and First Doses Reported to CDC



JYNNEOS Vaccine Effectiveness

Vaccine effectiveness of JYNNEOS against mpox ranges from 36%–75% for partial vaccination and 66%–86% for full vaccination

	Cases	Controls	Adjusted* VE (95% CI)
Partial vaccination (1 dose)			
Epic Cosmos case-control study	146	1000	36% (22–47)
Multi-jurisdictional case-control study	58	237	75% (61–84)
Full vaccination (2 doses)			
Epic Cosmos case-control study	25	335	66% (47–78)
Multi-jurisdictional case-control study	14	122	86% (74–89)



https://www.nejm.org/doi/full/10.1056/NEJMoa2215201?query=featured_home

https://www.cdc.gov/mmwr/volumes/72/wr/mm7220a3.htm?s_cid=mm7220a3_w

Vaccine effectiveness of JYNNEOS against mpox

- Further research needed to evaluate whether immunocompromised status modulates VE
- Further research needed to understand the duration of protection
- Break through infections have been observed

Is there need for additional smallpox vaccines?

- Safe and effective vaccines are a key component of smallpox preparedness
  - FDA-approved vaccines for use against smallpox
    - ACAM2000
    - Modified Vaccinia Ankara (JYNNEOS®)
- Concerns regarding both vaccines
  - Contraindicated for several populations (ACAM2000®)
  - Potential breakthrough infections with mpox (JYNNEOS®)
  - Having additional vaccines with different production methods would be beneficial

Use of *Variola virus* to support "novel" vaccine development - mRNA vaccine

- mRNA vaccines are safe and can be rapidly scaled up
- Moderna has developed an mRNA-based subunit vaccine targeting OPXVs
  - Encodes four OPXV antigens
  - Preliminary data in murine models indicate non-inferiority to MVA vaccine
    - Improved performance to MVA vaccine in some criteria
  - Dose-ranging study in NHPs completed
    - Humoral and cellular immune responses will be measured
- Moderna leading Phase I clinical trial in adults
  - Began August 2023 in U.K.
  - Humoral and cellular immune responses will be measured

Antivirals (4 approved VARV proposals)

TPOXX

ST-357

Monoclonal antibodies

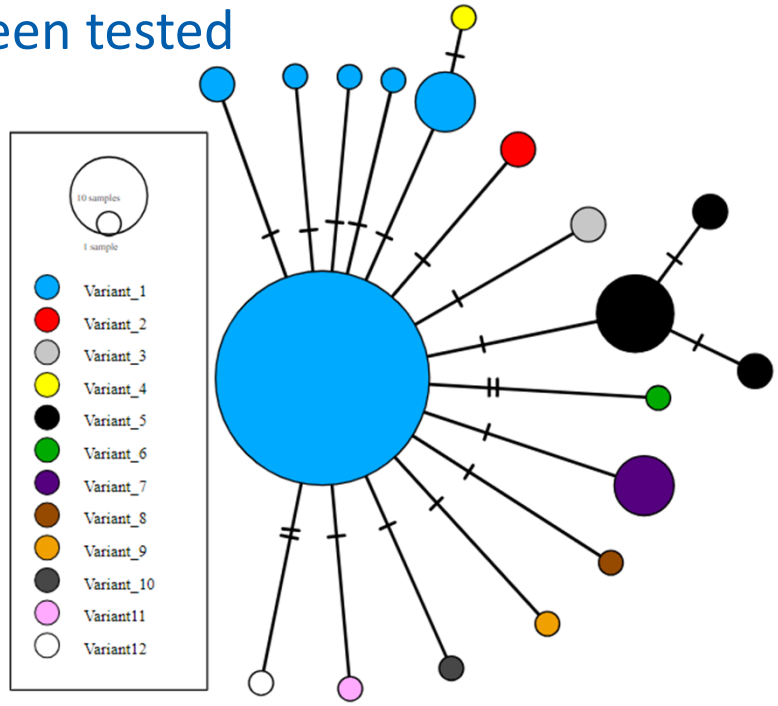
Small animal model

Antiviral therapeutics are a key public health need for smallpox preparedness

- Currently approved antiviral therapeutics for smallpox treatment
  - Tecovirimat (TPOXX®)
  - Brincidofovir (TEMBEXA®)
- Concerns about both drugs
  - TEMBEXA has safety concerns for some patients
  - Patients with mpox in which both drugs failed
  - Surveillance for drug resistance
- FDA Post Marketing Request to test expanded panel of VARV isolates for sensitivity to TPOXX

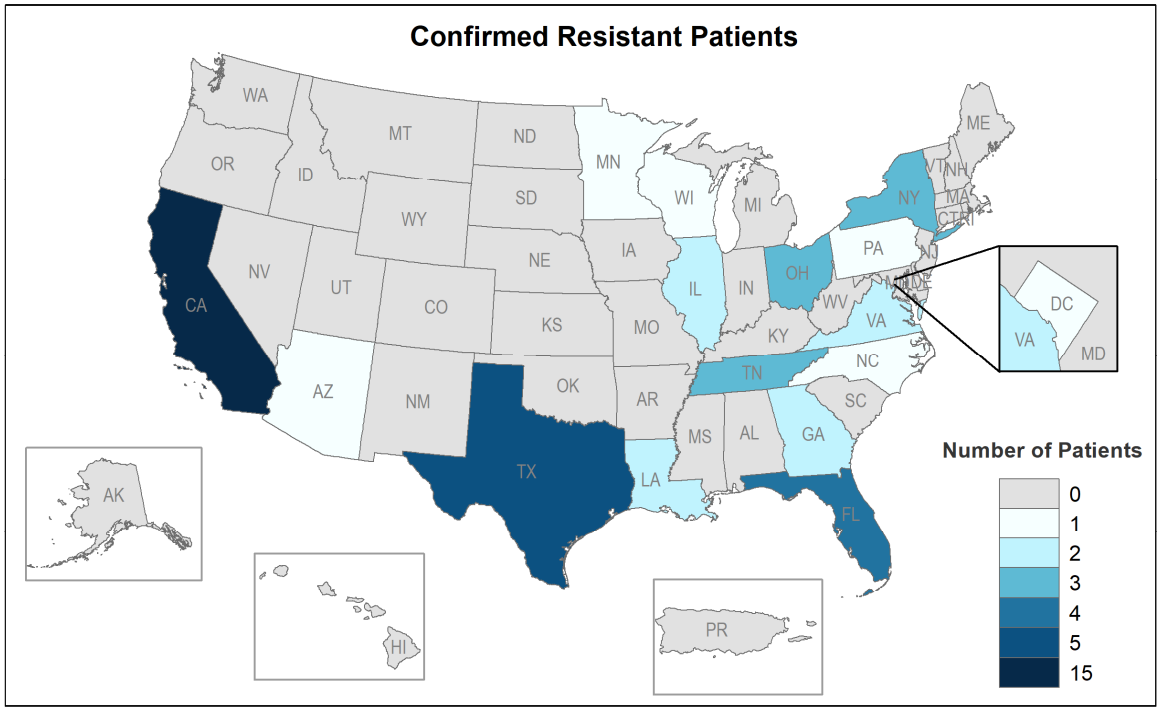
TPOXX for smallpox treatment

- TPOXX targets the viral protein F13
  - F13 is required for wrapping and egress of extracellular enveloped virus
- 8 of 12 VARV F13 amino acid variants have been tested
  - All were sensitive
- 19 nucleotide haplotypes
  - 12 amino acid variants
  - 2 new VARV F13 variants
    - These will be tested in 2024
- Data on VARV sensitivity to TPOXX submitted to FDA



TPOXX-resistance confirmed in mpox patients

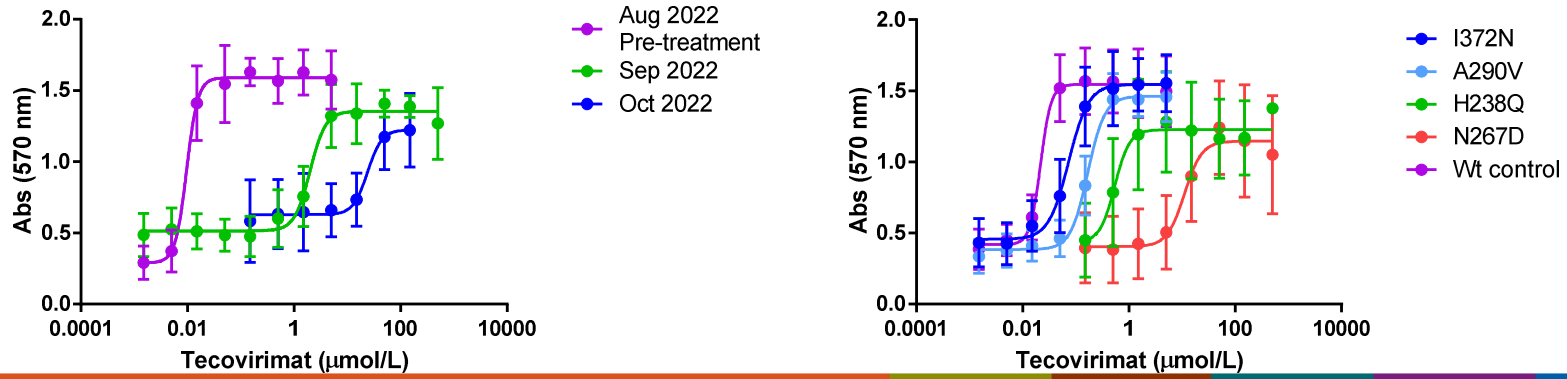
- 7563 patients treated
  - 435 submitted
  - 83 virus isolated
  - 68 phenotyped
  - 46 resistant
    - 39 HIV+
    - 31 uncontrolled
      - 10 deceased
    - 34 hospitalized
    - 39 treated



Smith, et al. EID, 2023

TPOXX-resistance in mpox patients

- Prolonged disease – average 51 days from diagnosis to resistant sample
- Prolonged treatment – average 39 days (14 to 167 days)
- Selected during treatment
  - Unique mutational profiles from same patient different sites
    - Different subpopulations selected at different sites
  - Longitudinal sampling



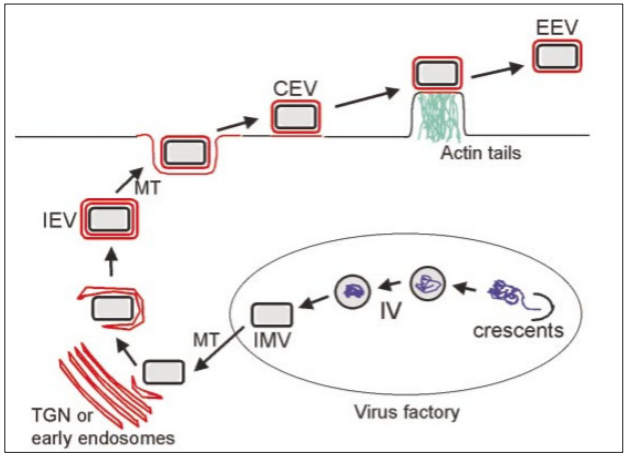
Smith, et al. EID, 2023

TPOXX discussion and conclusions

- Concerns about selection of drug-resistance
  - Frequency 1 – 4.5% of treated mpox patients
  - Higher in at-risk populations
  - Forward transmission occurs
- Additional smallpox antiviral therapeutics are needed

Monoclonal antibodies to treat smallpox

- Monoclonal antibody (mAb) treatments
  - 2 anti-*Zaire-ebolavirus* mAb products currently approved by Food and Drug Administration (FDA)
- Treatment options for Smallpox
  - TPOXX® (ST-246, tecovirimat): potential resistance
  - Tembexa (Brincidofivir): safety concerns for some populations
  - VIGIV: polyclonal Ab
    - Approved for treatments of smallpox vaccination complications
  - Best approach is multi-therapeutic
- Individual mAbs or cocktails could fill this gap
- Two primary forms of the virus
  - Intracellular mature virion (IMV)
  - Extracellular enveloped virion (EV)
- CDC has multiple collaborations with smallpox mAb developers



G.L. Smith, et al. Journal of General Virology (2002), 83, 2915-2931.

BioFactura Collaboration

- CDC began screening BioFactura mAbs against VARV in 2018
- In 2019 and 2023, BioFactura awarded Advanced Development Contract for its Smallpox Biodefense Therapeutic from BARDA
  - Originally composed of 3 mAbs: one directed against IMV and two directed against EEV
  - Ideally use a 2 mAb cocktail instead of 3
- Evaluating variations of the 2 mAb cocktail with variety of non-variola animal models with conflicting results
  - Variola PRNT assays demonstrated one of the mAbs did not neutralize well
    - The final 2 mAb combination was selected based in part on these results with live variola virus

BioFactura mAb Production & Ongoing Testing

- As mAbs move through production phases they are sent to CDC to confirm neutralization against live VARV
  - Neutralization compared to previous mAb iterations
- 2024: Continue testing of cocktails and mAbs to determine potential as MCMs
  - Efficacy of the final product needs to be re-confirmed against Variola *in vitro*
    - mAbs humanization and propagation modifications may impact efficacy

Acknowledgements

The Poxvirus and Rabies Branch- Past & Present
External Collaborators – Past & Present

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov