INTREPID ALLIANCE

INTERNATIONAL READINESS FOR PREVENTING INFECTIOUS VIRAL DISEASE

APRIL 7, 2025

DEEP DIVE REPORT

Antivirals for Orthopoxviruses: Focus on Mpox*

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ON BEHALF OF THE INTREPID ALLIANCE SCIENTIFIC WORKING GROUP

*BASED ON REVIEW OF PUBLIC DOMAIN INFORMATION AS OF MARCH 2, 2025

INTREPID Alliance. Antivirals for Orthopoxviruses: Focus on Mpox. 7 APRIL 2025. Available at intrepidalliance.org

Disclaimer

The INTREPID Alliance is a not-for-profit consortium of innovative biopharmaceutical companies committed to accelerating antiviral research, aiming to ensure that we have a stronger pipeline and are better prepared for future pandemics.

As part of our efforts, the INTREPID Alliance maintains and publishes a centralized list of promising investigational candidate compounds, with the purpose of knowledge-sharing and to support better pandemic preparedness. These compounds have been selected based on objective, scientific criteria, using publicly available sources, and at arm's length from commercial influence of our member companies. See criteria listed in the report "Antiviral Clinical Development Landscape and Promising Clinical Compounds." The designation of certain compounds as promising is based upon currently available information, and exclusively upon an assessment against these criteria. "Promising" is not a promotional claim. Candidate compounds have not been assessed by regulatory authorities to be safe and efficacious for the treatment of disease in humans. Our content is designed to be factual, informative, and non-commercial. It is not designed or intended to advertise or promote any pharmaceutical product or therapy or to advance the commercial interests of any company.

INTREPID Alliance Assessment of Mpox Direct-Acting Antivirals*

Topics:

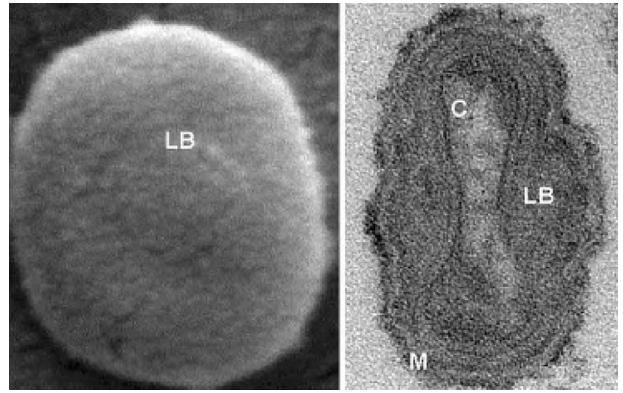
1. **Mpox Disease Landscape**
   - Orthopoxviruses (ORPVs) and human disease
   - Global picture of monkeypox virus (MPXV) outbreaks and mpox disease
   - Preparedness and response overview
2. **Mpox Clinical and Preclinical Landscape**
   - MPXV life cycle +/- direct-acting antiviral (DAA) sites of action
   - Clinical stage/Approved DAAs and highlights of available data
   - Representative preclinical small molecule DAAs under investigation
3. **Addressing the Unmet Need for MPVX/Orthopoxvirus Direct-Acting Antivirals**
   - Assessment of current and potential DAAs for mpox
   - Simplified roadmap for development of pan-ORPV DAAs
   - Recommendations to improve preparedness and response

*Based on review of public domain information as of March 2, 2025

Mpox Disease Landscape

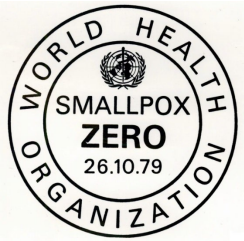
Orthopoxviruses and Humans

- ORPVs comprise a genus of Poxviridae that includes the human pathogens variola virus (VARV), monkeypox virus (MPXV), cowpox virus, camelpox virus, and vaccinia virus (VACV).
- ORPVs are morphologically and genetically similar; the conservation of many genes is reflected in the pan-ORPV activity of vaccines and approved antivirals.
- Smallpox disease (VARV) was one of the most consequential diseases in human history until its eradication in 1979.
- Disease from cowpox, camelpox, and VACV is uncommon and rarely severe.
- Mpox outbreaks (MPXV) in 2022 and 2024 were declared public health emergencies of international concern (PHEIC); second PHEIC reaffirmed in 2025.



Vaccinia virus: frontal and sagittal views. LB=lateral body; C=core; M=viral membrane

Gray RDM, et al. *Sci Rep* 2016; 6:29132.



ORPVs: orthopoxviruses.

CDC, Jan 2025; CDC, Oct 2024; WHO, History of the Smallpox Vaccine; WHO, A Brief History of Vaccines; Noyce, et al. 2018; McFadden, 2005; Ritter, et al. 2024; Titanji, et al. 2024.

Mpox: A Disease with Pandemic Potential

- Outbreaks may be initiated by zoonosis (e.g., rodent-human) followed by human-human spread.
- Major mode of human transmission is direct skin-skin contact.
  - Transmission is possible before symptoms, but most spread occurs after symptoms.
- MPXV clades/subclades/strains may differ in ease of transmission and clinical severity.
- Incubation period is 2-21 days, followed by prodromal symptoms (e.g., fever, headache, malaise) for 1-5 days before lesions appear.
- Lesions progress from macules to papules to vesicles to pustules before scabbing; lesions typically heal in 2-4 weeks.
- Mortality is <0.2% to 10% depending on clinical care, patient vulnerability, and virulence of MPXV strain.

MPXV: monkeypox virus.

Titanji, et al. 2024.

Differences in Clinical Disease Among Relevant MPXV Clades/Subclades

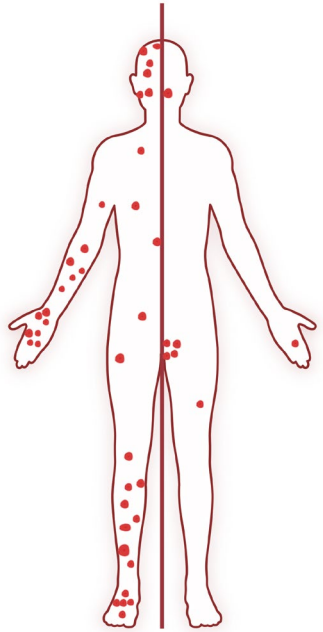
Clade I (PHEIC 2024-present)

Case fatality rate <1-10%

- Ia > Ib
- Majority of deaths in children <5 years of age
- Ib in 14 countries outside Africa*

Lesions often widespread (centrifugal rash, spreading outward from a central point)

Human-human infection common among household contacts; ≈1/3 are children



Clade II (PHEIC 2022-2023)

Case fatality rate <1%

- IIb reported in >120 countries
- >100,000 cases worldwide
- >33,000 cases/60 deaths in U.S.

Fewer lesions; anogenital concentration

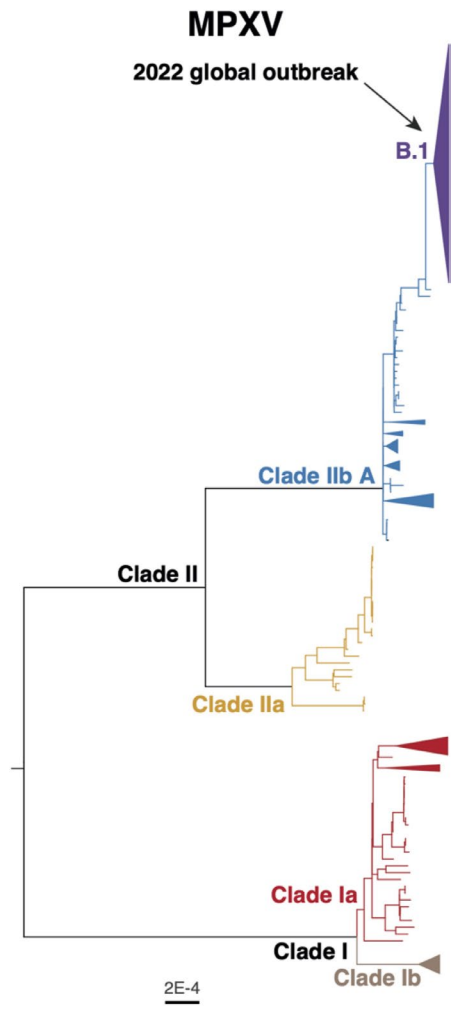
Human-human infection associated with sexual activity among men

*As of March 2, 2025

MPXV: monkeypox virus; PHEIC: public health emergency of international concern.

Titanji, et al. 2024; Vakaniaki, et al. 2024; Rivers, et al. 2024; O'Toole, et al. 2023; Kinganda-Lusamaki, et al. 2025; WHO, Multi-country outbreak of mpox. External situation report #48.

Epidemiology of Mpox by MPXV Clade



Phylogenetic tree of all MPXV clades
World Health Organization. Global Mpox Trends – Key Figures, March 2025.

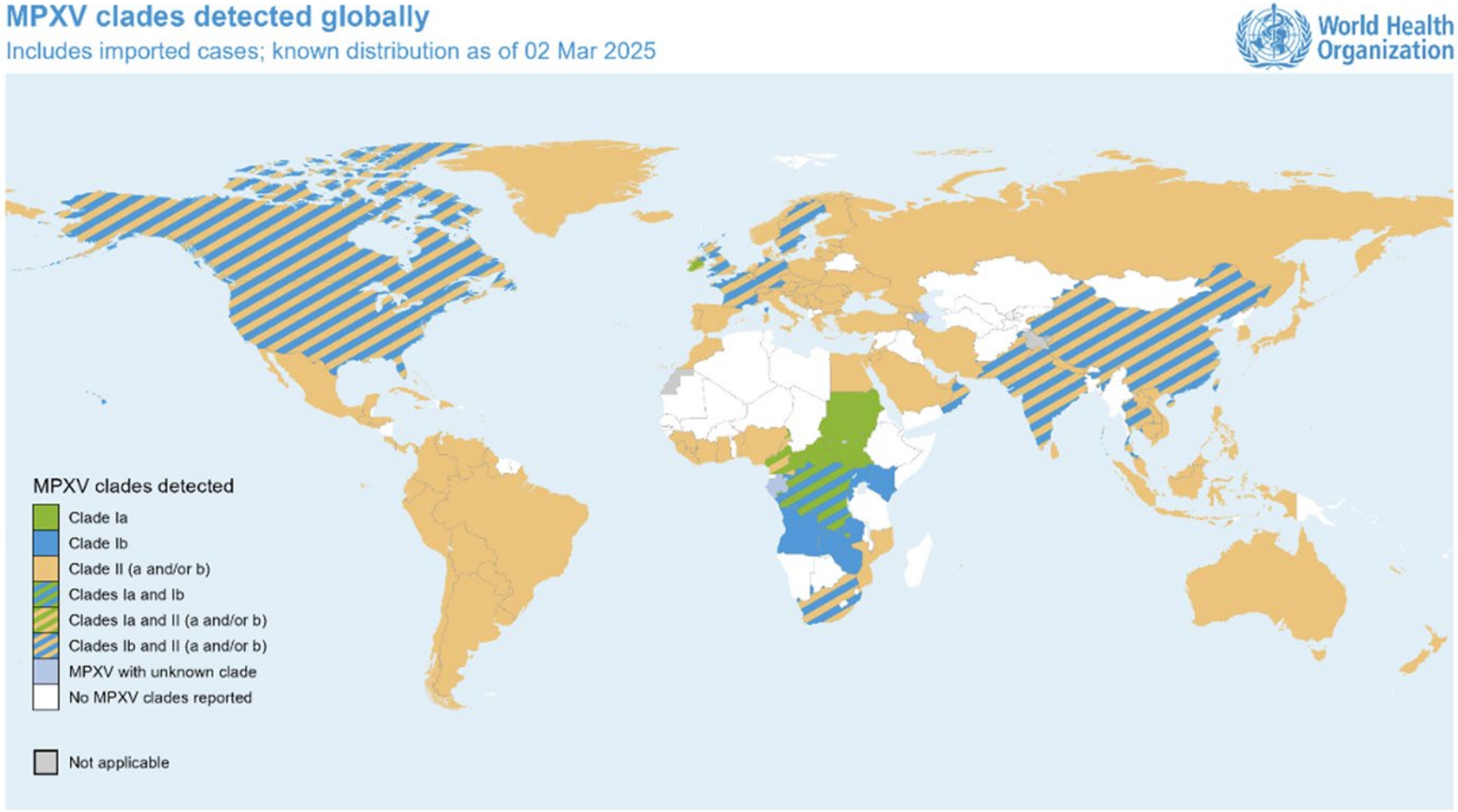
- Clade IIb primary strain in PHEIC 2022-2023
- >100,000 infections worldwide with >33,000 being in U.S.
- Continued spread with local outbreaks in 2024 (e.g., ≈200 new infections/month in U.S.)
- Spread of antiviral (tecovirimat) resistant strains documented

- Clade Ib primary strain in PHEIC 2024
- ≈62,000 cases and >1,200 suspected deaths in Africa in 2024
- Confirmed 1b cases in 14 countries outside of Africa as of March 2, 2025

MPXV: monkeypox virus; PHEIC: public health emergency of international concern.

WHO, Multi-country outbreak of mpox. External situation report #48; Titanji, et al. 2024; Miura, et al. 2024; CDC, Nov 2024; Delea, et al. 2024; Vakaniaki, et al. 2024; Rivers, et al. 2024; Kinganda-Lusamaki, et al. 2025; Univ of MN, CIDRAP, 2024.

Global Distribution of Mpox by MPXV Clade (March 2, 2025)



The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area of its authorities, or concerning the delimitation of its frontiers or boundaries.
Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: WHO Health Emergencies Programme

MPXV: monkeypox virus.

WHO, Multi-country outbreak of mpox. External situation report #48.

Medical Countermeasure Summary

- **Diagnostics:**
  - qPCR assays approved; need rapid (sensitive & specific) point-of-care tests.
  - Ideally tests should identify clade/subclade as clinical prognosis/treatment may be different.
- **Vaccines: Disease prevention/mitigation**
  - Live, attenuated vaccines have rare, but potentially serious side effects which limit use.
  - Non-replicating vaccines safer (and more expensive); efficacy is ≈66% to 86% but wanes over time.
- **Therapeutics: Disease prevention/treatment (most common)**
  - Therapeutic vaccination must be within a few days of exposure.
  - Monoclonal antibodies (mAbs) are promising; challenges may include cost, cold-chain requirement, injectable formulation, emergence of resistant strains.
  - DAAs have potential to be relatively inexpensive, oral, temperature stable, and have higher barriers to resistance.
  - DAAs are focus of this presentation.

DAAs: direct-acting antivirals.

CDC, Feb 2025; WHO, Sep 2024; WHO Advisory Committee, 2024; Titanji, et al. 2024.

Mpox Clinical and Preclinical Landscape

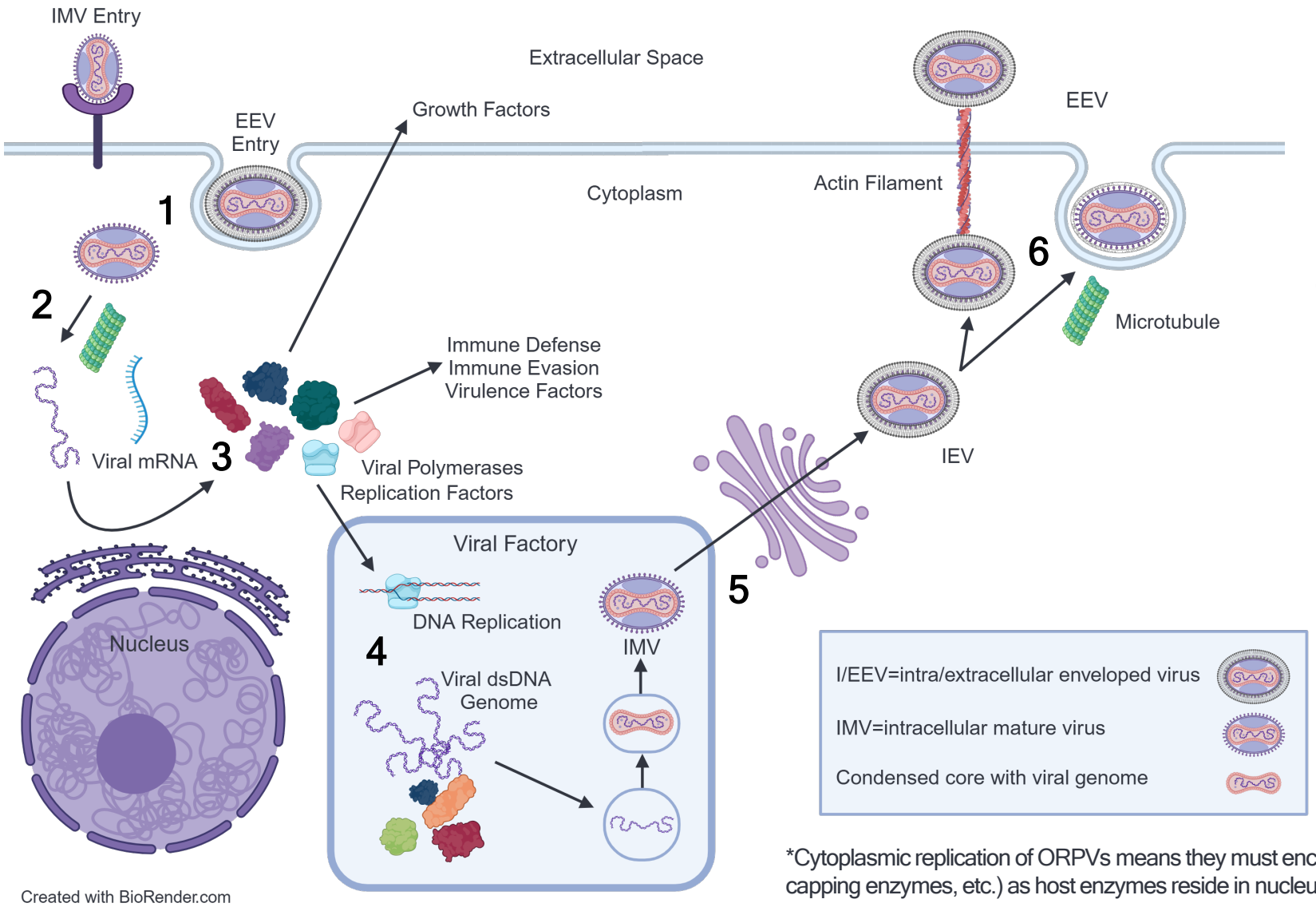
Overview of Direct-Acting Antivirals for Mpox/Orthopoxvirus Disease

- **Clinical**
  - ORPVs have pandemic potential; DAAs are needed to meet the 100 Days Mission.
  - Two pan-ORPV DAAs in Phase 3 clinical development have known limitations.
    - Tecovirimat has a low barrier to resistance and failed two recent mpox trials (PALM007 and STOMP).
    - Brincidofovir has known safety concerns that may limit dosing/utility.
- **Roadmap for DAA discovery/development**
  - The ORPV life cycle depends on conserved viral proteins which are good targets for DAA discovery.
  - Small molecules with MPXV activity have been identified; some may be tractable leads.
  - Development pathways for DAAs are well-understood; timelines are typically longer than other medical countermeasures (diagnostics, vaccines, mAbs).

ORPVs: Orthopoxviruses; DAAs: direct-acting antivirals; MPXV: monkeypox virus; mAbs: monoclonal antibodies.

Titanji, et al. 2024; FDA, Center for Drug Evaluation and Research, Mar 2025; NIH, Aug 2024; NIH, Dec 2024; Chan-Tack, et al. 2021; IPPS, 100 Days Mission.

MPXV/Orthopoxvirus Life Cycle



1. MPXV enters target cells
2. Microtubule transport to perinuclear region and uncoating
3. Early protein expression
4. Synthesis of new MPXV genomes and additional protein expression*
5. IMV enveloped in Golgi to form IEV
6. Transport to the cell surface via actin or microtubules

*Cytoplasmic replication of ORPVs means they must encode their own proteins for DNA and RNA replication (polymerases, capping enzymes, etc.) as host enzymes reside in nucleus.

Created with BioRender.com

MPXV: monkeypox virus.

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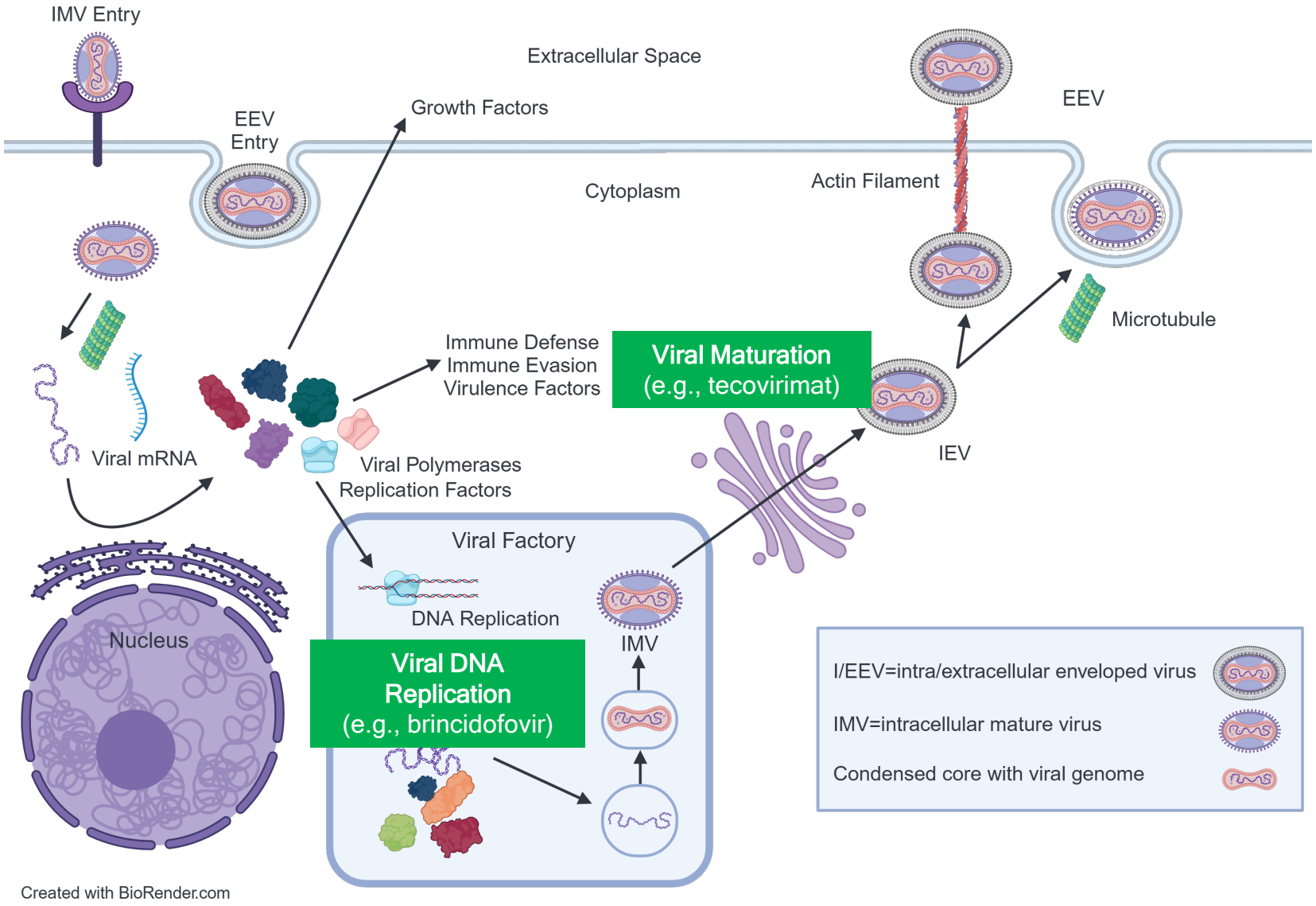
MPXV Life Cycle and Small Molecule Direct-Acting Antivirals in Clinical Trials



- Tecovirimat inhibits viral maturation
  - Target: Viral protein 37
- Brincidofovir inhibits viral DNA replication
  - Target: Viral DNA polymerase

MPXV: monkeypox virus.

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Clinical Stage Direct-Acting Antivirals for Mpox*

Maturation

Tecovirimat (oral; IV)

- Adults and pediatric
- Oral tablet, twice daily for 14 days
- Approved for Smallpox (U.S., EU, Japan)
- Approved for mpox (EU, Japan)
- Expanded Access-Investigational New Drug (EA-IND) protocol for mpox (U.S.)

NIOCH-14 (oral; Russia only); active antiviral identical to tecovirimat

Replication

Brincidofovir (oral; IV in development)

- Adults and pediatric
- Oral tablet/suspension, once weekly for two doses
- Approved for Smallpox (U.S., Canada)
- FDA-authorized single-patient emergency use IND (e-IND) for mpox (U.S.)

Trifluridine (topical for ophthalmic use); not used systemically for mpox

- Effectively, two oral antiviral treatment options for mpox (tecovirimat and brincidofovir)
- Clinical trials of these two completed and/or ongoing in Africa and outside of Africa
- Need additional/improved oral DAAs with complementary mechanisms of action (MOAs)

*In Phase 2 or Phase 3 trials, approved for an ORPV, and/or in clinical use (ophthalmic Trifluridine) as of December 2024; ASC10 (prodrug of molnupiravir) has had an open IND for mpox for >2 years but there is no evidence a trial for mpox has been initiated as of March 2, 2025.

DAAs: direct-acting antivirals; ORPVs: Orthopoxviruses; IND: Investigational New Drug.

Cohen, Jan 2025; Titanji, et al. 2024; Ascletis Pharma Inc., Nov 2022.

Completed Clinical Trials for MPXV Direct-Acting Antivirals

Data Limited to Tecovirimat

- The largest observational study with tecovirimat (N=7,181 patients) concluded: *"The EA-IND data are not definitive; controlled clinical trial data are essential to elucidating if and how tecovirimat should be used."*[1]
- One RCT (PALM007; N=597 patients in the DRC with clade I MPXV) showed no difference among placebo and tecovirimat treated patients for the primary endpoint (time to lesion resolution).
  - *"The antiviral drug tecovirimat did not reduce the duration of mpox lesions among children and adults with clade I mpox…"*[2]
  - No clear explanation yet for apparent lack of effect in overall population, but important data pending (e.g., PK, resistance) and Siga has stated there is some benefit for patients treated early who had >100 lesions (although not significant)[3]
  - CFR 1.7% in both arms
- A second RCT (STOMP; international, clade II MPXV) found "*tecovirimat did not reduce the time to lesion resolution or have an effect on pain among adults with mild to moderate clade II mpox*"[4]

MPXV: monkeypox virus; EA-IND: expanded access investigational new drug; RCT: randomized controlled trial; DRC: Democratic Republic of the Congo; PK: pharmacokinetic; CFR: case fatality rate.

[1]Yu, et al. 2024; [2]NIH, Aug 2024; [3]Cohen, Jan 2025; [4]NIH, Dec 2024; IDSE, Jan 2025.

Completed and Ongoing Randomized Controlled Trials of Direct-Acting Antivirals for Mpox*

DAA	Study Type	Study Name	Sponsor	Location	MPXV Clade	Target Sample Size	Status / Completion**	ID #
Tecovirimat	Phase 2 RCT	PALM007	NIAID	DRC	Clade I	450; actual 597	Completed 2024-09-03	NCT05559099
Brincidofovir	Phase 3 RCT	MOSA	PANTHER	DRC+	Any	422	Recruiting	
Tecovirimat	Phase 3 RCT	UNITY	ANRS	Brazil, Argentina, Switzerland	Clade IIb	150	Recruiting; 2026-08-01	NCT05597735
Tecovirimat	Phase 3 RCT	STOMP	NIAID	USA+	Clade IIb	530; actual 719	Enrollment stopped	NCT05534984
Tecovirimat	Phase 3 RCT	PLATINUM-CAN	McGill University	Canada	Clade IIb	120	Recruiting; 2025-03	NCT05534165
Tecovirimat	Phase 4 RCT	EPOXI	UMC Utrecht ECRAID	EU	Clade IIb	150	Recruiting; 2026-8	NCT06156566; 2022-501979-10

*Randomized controlled clinical trials as of Q4 2024; **Estimated completion

DAA: direct-acting antiviral; RCT: randomized controlled trial; NIAID: National Institute of Allergy and Infectious Diseases; PANTHER: PANdemic preparedness plaTform for Health and Emerging infectious Response; ANRS: Agence Nationale de Recherches sur le Sida; ECRAID: European Clinical Research Alliance for Infectious Diseases; DRC: Democratic Republic of the Congo; DRC+: DRC and surrounding countries; MPXV: monkeypox virus.

Fox, et al. 2023; Olliaro, et al. 2024; ClinicalTrials.gov; NIH, Aug 204; IDSE, Jan 2025; PANTHER, Nov 2024.

Summary of Clinical Stage MPXV/Orthopoxvirus Small Molecule Direct-Acting Antivirals (Q4 2024)

- Two pan-ORPV small molecule DAAs (tecovirimat and brincidofovir) are in clinical trials for mpox.
  - Tecovirimat failed to meet the primary endpoint (reduction in duration of mpox lesions/pain) in two large RCTs.
    - PALM007, N=597, clade I and STOMP, N=719, clade II
  - Brincidofovir Phase 3 RCT initiated (MOSA); data anticipated mid-2025
- ASC10 (molnupiravir prodrug) IND is available for mpox; no report of an ongoing trial.
- Novel small molecule DAAs are needed to meet 100 Days Mission to optimize preparedness for current/potential ORPV outbreaks.

MPXV: monkeypox virus; ORPVs: Orthopoxviruses; DAAs: direct-acting antivirals; RCT: randomized controlled trial; MOSA: MpOx Study in Africa; IND: Investigational New Drug.

Yu, et al. 2024; NIH, Aug 2024; NIH, Dec 2024; Ascletis Pharma Inc., Nov 2022; Africa CDC, 2025 ; IPPS, 100 Days Mission.

Preclinical MPXV/Orthopoxvirus Small Molecule Direct-Acting Antivirals (Q4 2024)

- ORPVs have several hundred genes; many are highly conserved and essential to virus replication, providing good targets for discovery of pan-ORPV DAAs.
- Preclinical stage DAAs have been identified as potential antivirals for mpox.
  - Some are novel compounds, and some are repurposed (approved or investigational) drugs.
  - The most promising are being evaluated by INTREPID Alliance using its preclinical triage and target compound profile.

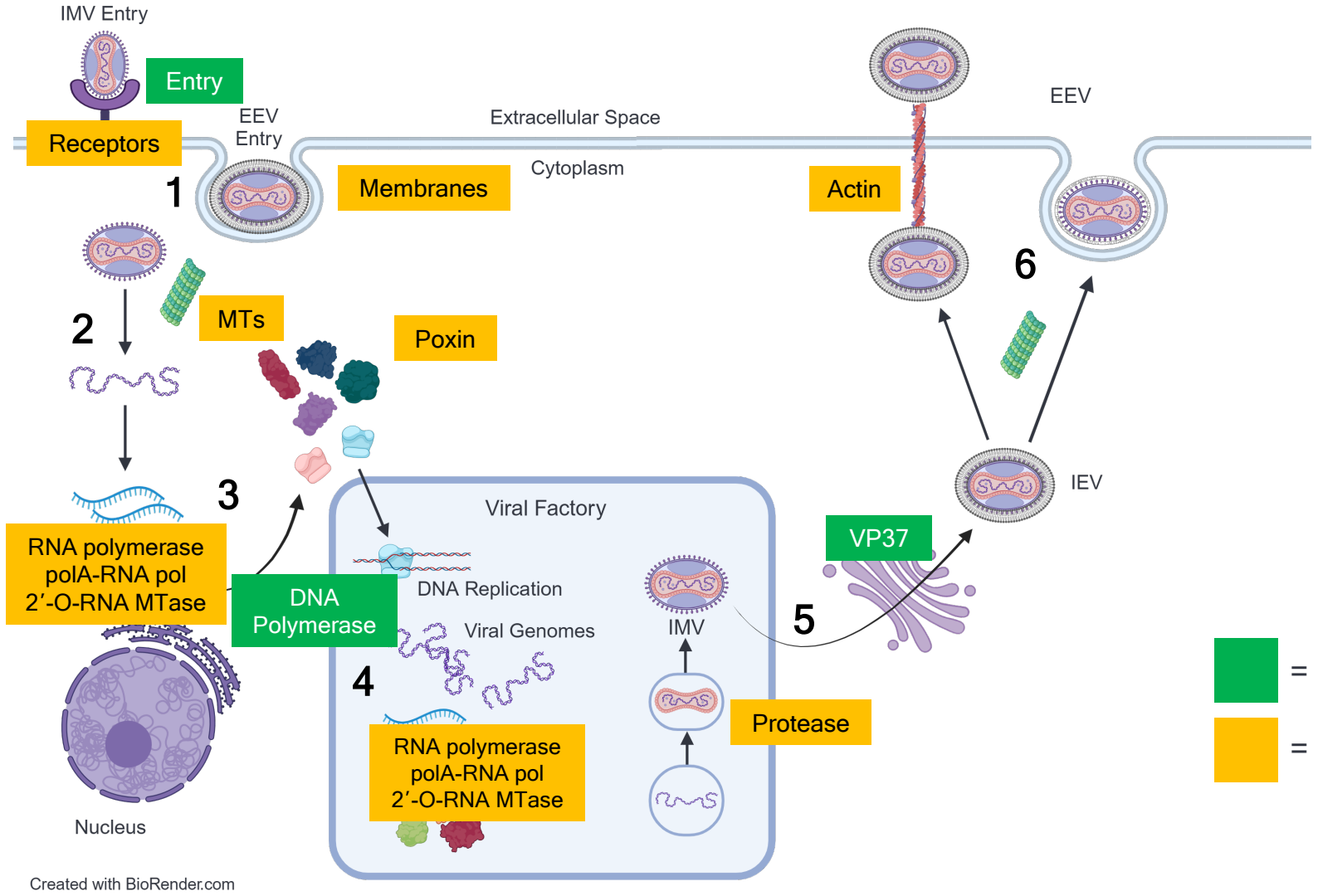
MPXV: monkeypox virus; ORPVs: Orthopoxviruses; DAAs: direct-acting antivirals.

Key Preclinical Attributes and Activities for Advancing an Antiviral Compound Profile

Chemistry, manufacturing and controls not included for simplicity given stage(s) of development

Key Attribute	Preclinical Attributes and Activities
Potency and Efficacy	• Mechanism of action • Spectrum of *in vitro* antiviral activity; virus of interest; relevant clinical isolates, and activity within and outside the viral family • *In vivo* efficacy in relevant animal model: prophylactic and/or treatment design based on target product profile • Resistance and cross-resistance
Preclinical Pharmacology	• Pharmacokinetic/Pharmacodynamic relationship • Human pharmacokinetics and dose prediction • Metabolism, clearance and target tissue distribution • Drug-drug interactions: low/no cytochrome P450 (CYP) enzyme induction or inhibition, low/no time-dependent CYP inhibition
Preclinical Safety	• Review regulatory guidance to inform requirements and experimental design(s) • *In vivo* studies (single/repeat dose toxicity, metabolites) • *In vitro* studies (genotoxicity, cytotoxicity, off-target activity, human hERG (Ether-a-go-go-Related Gene), BSEP (bile salt export pump)
Use in Combination Antiviral Therapy	• *In vitro* drug combination assays based on clinical relevance of the regimen components and potential benefit for synergy and barrier to resistance (differentiated from drug-drug interactions)

Examples of Targets for MPXV Direct-Acting Antivirals Discovery



MPXV/ORPV Life Cycle

1. Viral entry
2. Transport/Uncoating
3. Early protein expression
4. Viral DNA synthesis, protein synth./processing
5. Maturation
6. Transport/Release

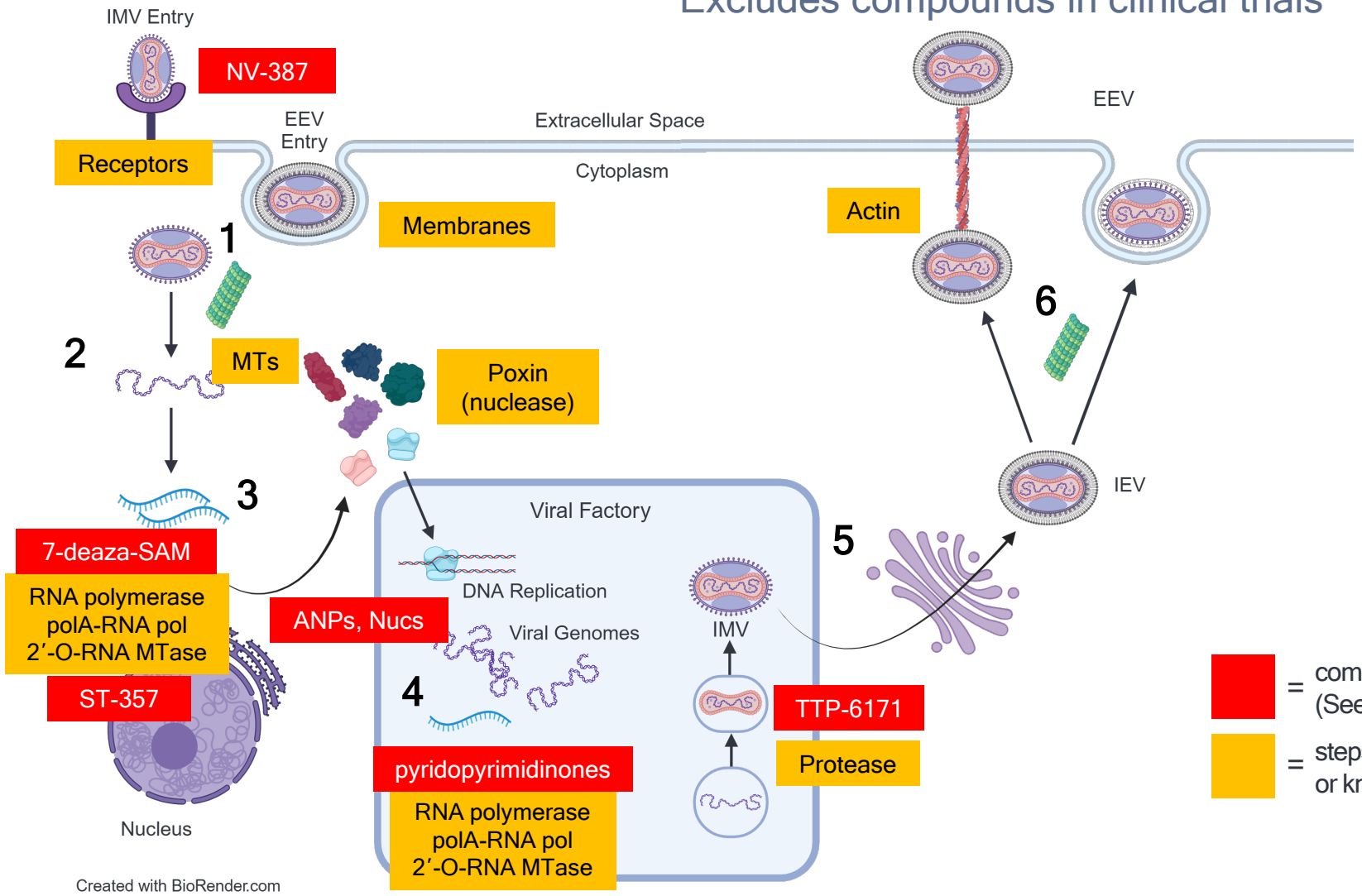
= targets of current clinical compounds

= steps with conserved targets, but no ORPV precedent or known/likely issues

MPXV: monkeypox virus; ORPV: Orthopoxvirus; EEV: extracellular enveloped virus; IMV: intracellular mature virus.

Examples of Direct-Acting Antivirals Hits and Leads for Mpox

Excludes compounds in clinical trials



MPXV/ORPV Life Cycle

1. Viral entry
2. Transport/Uncoating
3. Early protein expression
4. Viral DNA synthesis, protein synth./processing
5. Maturation
6. Transport/Release

= compound with activity against this step of life cycle (See slide 23 for detail on compounds)

= steps with conserved targets, but no ORPV precedent or known/likely issues

MPXV: monkeypox virus; ORPV: Orthopoxvirus; EEV: extracellular enveloped virus; IMV: intracellular mature virus; ANP: acyclic nucleoside phosphonate; MTase: methyltransferase; SAM: S-adenosylmethionine

See slide 23 for references and upcoming 4th Edition of the INTREPID Alliance Antiviral Landscape; See Glossary for ‘Hit’ and ‘Lead’ definitions.

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Conserved MPXV/Orthopoxvirus Targets and Examples of Direct-Acting Antivirals (February 28, 2025)*

ORPV Target	Compound(s)	Developer	Stage	Key References
DNA polymerase	**Brincidofovir**; Adefovir; HPMPDAP (diaminopurine); 5-iodo-2-deoxyuridine	Texas A&M, CDC, Academy of Sciences of the Czech Republic, Rega Institute, Gilead Sciences	Early Leads to Clinical Candidate	Chan-Tack K, et al. *Antiviral Res.* 2021; 195:105182. Dsouza L, et al. *Antiviral Res.* 2023; 216:105651. Krečmerová M, et al. *J. Med. Chem.* 2010; 53:6825-6837. Neyts J, et al. *Antimicrob. Agents Chemother.* 2002; 46.
RNA 2′-O methyltransferase	7-deaza analogs of S-adenosyl methionine	Academy of Sciences of the Czech Republic	Hit-Lead (cell permeability optimization needed)	Zgarbová M, et al. *Antiviral Res.* 2023; 218:105714.
mRNA poly-A polymerase	ST357(TTP-018)	Siga Technologies	Early Lead (solubility optimization needed)	WHO Advisory Committee on Variola Virus Research: report of the twenty-fifth meeting, 2023.
I7L-proteinase	TTP-6171	Siga Technologies	Hit (potency optimization required)	Byrd CM, et al. *J. Virol.* 2004; 78(22). Dodaro A, et al. *Int. J. Mol. Sci.* 2023; 24, 7119. Imran M, et al. *Biomedicines* 2024; 11(7), 2025.
Virus attachment	NV-387	Nanoviricide Inc.	Clinical candidate	Chakraborty A, et al. *PLoS One.* 2022; 17(12). Company website.
RNA polymerase (presumptive)	ASC10, a prodrug of molnupiravir.	Ascletis Pharma Inc.	IND open for mpox	Liu J, et al. *Exp. Op. Inv. Drugs* 2024; 33(8):867-876. ClinicalTrials.gov.
RNA polymerase	CMLDBU6128 and improved pyridopyrimidinones	Boston University, CDC	Early Leads being optimized for potency/DMPK	Brown LE, et al. *Antimicrob Agents Chemother.* 2022;66(11):e0084122.
DNA-dependent RNA polymerase (presumptive)	UMM-766 (7-fluoro-7-deaza-2 2′-C-methyladenosine)	USAMRIID, Merck & Co.	Late Lead; MOA for mpox still unproven	Mudhasani RR, et al. *Microbiol Spectr.* 2024;12(4):e0358623.
Viral protein 37	**Tecovirimat; NIOCH-14**	Siga Technologies; Russia	Phase 3/approved	Chan-Tack, K, et al. *Lancet ID.* 2019; 19(6). Merchlinsky M, et al. *Antiviral Res.* 2019; 168:168-174.

Bold = clinical candidate

*Does not include hits from virtual screens; compounds shown are known or likely “pan-ORPV” inhibitors (based on homology).

MPXV: monkeypox virus; See Glossary for definitions for stages.

Overview of Small Molecule Direct-Acting Antivirals for MPXV/Orthopoxviruses

Downside	Upside
• DAAs currently in late-stage clinical development for MPXV/ORPVs have known limitations. • The dramatic rise in mpox cases/fatalities from 2020-2024 emphasizes a gap in preparedness for mpox/ORPV pandemics. • DAAs cannot be developed at the same speed as vaccines/mAbs. • Current DAAs do not meet the 100 Days Mission goals for mpox (or any ORPV).	• Current DAAs may work well together and provide a bridge to more optimized care. • Multiple, highly conserved, essential proteins support pan-ORPV discovery. • Preclinical stage DAAs have been identified as potential antivirals for mpox. • Development pathways for novel pan-ORPV DAAs are well understood and supporting resources are available as described in the next section.

MPXV: monkeypox virus; DAAs: direct-acting antivirals; ORPVs: Orthopoxviruses; mAbs: monoclonal antibodies.

IPPS, 100 Days Mission.

Addressing the Unmet Need for MPVX/Orthopoxvirus Direct-Acting Antivirals

Continuing Need for MPXV/Orthopoxvirus Direct-Acting Antivirals

- MPXV and other ORPVs have pandemic potential.
- Clinical trials with current DAAs (tecovirimat and brincidofovir) are ongoing and may not completely address the unmet need.
  - Tecovirimat failed two RCTs for mpox (one for clade I and one for clade II) in 2024.
  - Brincidofovir initiated a mpox RCT in late 2024.
- Additional DAAs are needed to meet the 100 Days Mission benchmark and enhance pandemic preparedness for ORPVs.
  - Ideally need at least two orally delivered, well-tolerated, effective pan-ORPV DAAs* with distinct mechanisms of action and different resistance profiles.

*pan-ORPV DAA defined as a small molecule antiviral with demonstrated or predicted efficacy against all orthopoxviruses known to cause human disease.

MPXV: monkeypox virus; ORPVs: Orthopoxviruses; DAAs: direct-acting antivirals; RCT: randomized controlled trial; mAbs: monoclonal antibodies.

IPPS, 100 Days Mission.

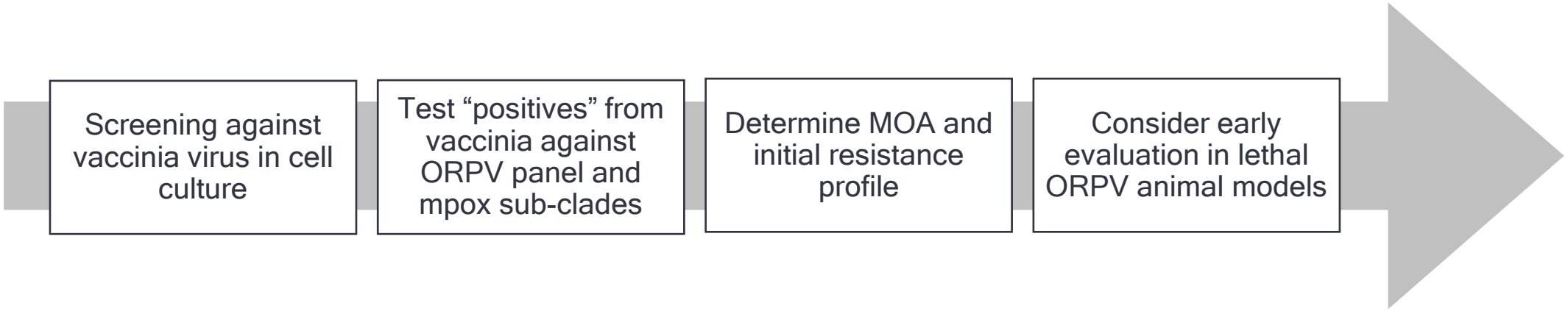
Meeting the Need: Reasons for Optimism

- The large genomes of ORPVs encode highly conserved proteins that are essential to viral replication and are targets of existing DAAs and/or hits from cell-based screens.
  - DNA and RNA polymerases
  - Structural proteins involved in maturation/viral egress
  - Methyltransferases
  - Proteases
- Pan-ORPV hits discovered; require medicinal chemistry optimization (e.g., for potency, selectivity, bioavailability).
- Academic and government expertise are available to drive ORPV DAA development.
  - NIH, CDC, BARDA, ORPV virology experts who have helped develop existing DAAs
  - Multiple animal models available at multiple sites (contract, government, academic)
- Development pathways for novel ORPV DAAs are well understood and relatively straight forward.

ORPVs: Orthopoxviruses; DAAs: direct-acting antivirals; NIAID: National Institute of Allergy and Infectious Diseases; CDC: Centers for Disease Control and Prevention; BARDA: Biomedical Advanced Research and Development Authority.

INTREPID Alliance Simplified Hit-to-Lead Roadmap

- Use high-throughput and/or focused library (e.g., protease inhibitors or nucleosides) screening with cell-based assays to generate chemical leads for optimization.
- Consider leveraging computational/modeling/AI approaches through cryo-electron microscopy/X-Ray structures of the viral target +/- ligand to accelerate the optimization process.
- Optimize compound chemistry to improve potency, selectivity, and bioavailability.



Cryo-EM: cryo-electron microscopy; AI: artificial intelligence; ORPV: Orthopoxvirus; MOA: mechanism of action. See Glossary for ‘Hit’ and ‘Lead’ definitions.

INTREPID Alliance, Antiviral Clinical and Preclinical Development Pipeline; NIAID, Mar 2022; Virology Research Services, Aug 2019; Virology Research Services, Sep 2019; Virology Research Services, Oct 2019; Rosa, et al. Jan 2023; Sun, et al. Jul 2022; BioCurate, Mar 2025.

Considerations for Animal Models in MPXV/Orthopoxvirus Antiviral Development

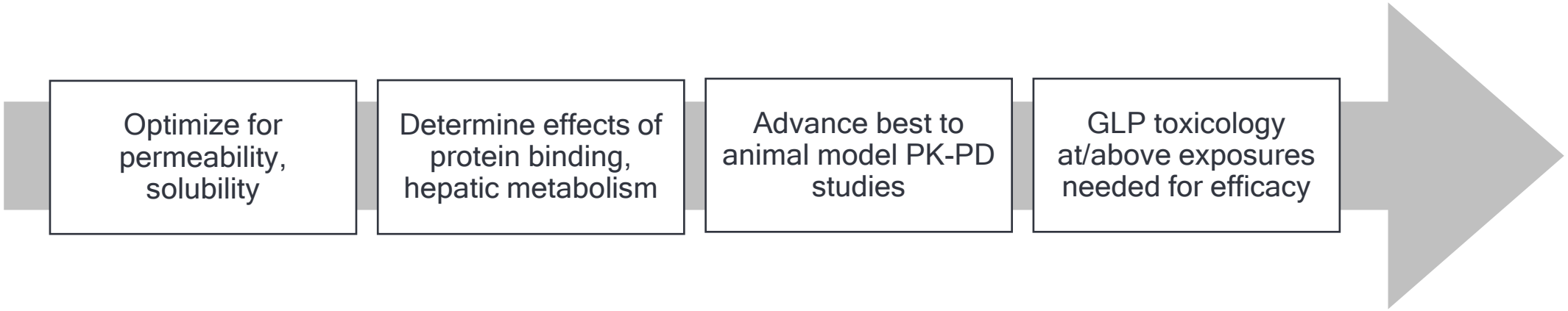
- Many ORPV animal models have been used to study various aspects of these infections.
  - For example, >20 models for mpox described in one manuscript.[1]
- Tightly controlled models were used for animal rule approval of tecovirimat (monkey and rabbit) and brincidofovir (mouse and rabbit) as treatments for smallpox.
  - Each of these models needed to show consistent, reproducible results at two centers.
- Myriad factors affect the pathophysiology of these models (e.g., species, animal age, immune status, virus species, inoculum, inoculation site).
  - ORPVs encoding multiple proteins involved in immune evasion; this is often host species-specific.
- Animal model(s) for a given question/aim should be chosen following expert advice.

MPXV: monkeypox virus; ORPV: Orthopoxvirus.

[1]Wei, et al. Sep 2023; Americo, et al. Feb 2023; Rosa, et al. Jan 2023.

INTREPID Alliance Simplified Lead-Candidate Roadmap

- Optimize compounds for permeability, solubility, human plasma protein binding and hepatic metabolism to identify orally bioavailable compounds that are likely to retain potency *in vivo*.
- Establish the PK-efficacy (or PK-PD) relationship in proof-of-concept animal models for high-value leads to predict target exposures/dosing regimens for clinical development.
- Early CMC assessment of cost of goods, stability, formulation, etc.



PK: pharmacokinetic; PD: pharmacodynamic; CMC: chemistry, manufacturing, and controls; GLP: Good Laboratory Practice. See Glossary for 'Lead' definition.

INTREPID Alliance, Antiviral Clinical and Preclinical Development Pipeline; NIAID, Mar 2022; Virology Research Services, Aug 2019; Virology Research Services, Sep 2019; Virology Research Services, Oct 2019; Rosa, et al. Jan 2023; Sun, et al. Jul 2022; BioCurate, Mar 2025.

Recommendations for Clinical Development of MPXV/Orthopoxvirus Direct-Acting Antivirals

- Research and develop novel antivirals, emphasizing orally-delivered, pan-ORPV DAAs with different MOAs and resistance profiles
  - Evaluate combination therapies of DAAs early in development
- Avoid using DAAs with low barriers to resistance as monotherapy in treatment of active disease as high viral burden amplifies the risk for selection of resistance
  - Monotherapy use of DAAs with low resistance barriers may be acceptable for prevention/post-exposure prophylaxis due to absent/low viral burden
- Conduct rigorous randomized, controlled clinical trials for ORPVs currently causing disease in humans as early as possible

MPXV: monkeypox virus; DAAs: direct-acting antivirals; MOAs: mechanisms of action.

Glossary of Terms

- **ADME:** absorption, distribution, metabolism, and excretion
- **AI:** artificial intelligence
- **ANP:** acyclic nucleoside phosphonate
- **ANRS:** Agence Nationale de Recherches sur le Sida
- **Approved Antiviral-Indication Expansion**: antiviral approved for one or more viral disease indications (e.g., cidofovir, favipiravir, molnupiravir, remdesivir, valganciclovir)
- **BARDA:** Biomedical Advanced Research and Development Authority is a center within the Administration for Strategic Preparedness and Response (ASPR) located within the U.S. Department of Health and Human Services (HHS)
- **CDC:** Centers for Disease Control and Prevention
- **CFR:** case fatality rate
- **CMC:** chemistry, manufacturing, and controls
- **Cryo-EM:** cryo-electron microscopy
- **DAA:** direct-acting antiviral
- **DRC:** Democratic Republic of the Congo
- **EA-IND:** expanded access investigational new drug
- **ECRAID:** European Clinical Research Alliance for Infectious Diseases

Glossary of Terms (cont'd)

- **EEV:** extracellular enveloped virus
- **FIH**: first-in-human
- **GLP:** Good Laboratory Practice
- **IEV:** intracellular enveloped virus
- **IMV:** intracellular mature virus
- **IND:** Investigational New Drug
- **mAbs**: monoclonal antibodies
- **MOA:** mechanism of action
- **MOSA:** MpOx Study in Africa
- **Mpox:** disease caused by monkeypox virus
- **MPXV:** monkeypox virus
- **MTase:** methyltransferase
- **NIAID:** National Institute of Allergy and Infectious Diseases
- **NIH:** National Institutes of Health
- **ORPV:** Orthopoxviruses

Glossary of Terms (cont'd)

- **pan-ORPV DAA:** small molecule antiviral with demonstrated or predicted efficacy against all orthopoxviruses known to cause human disease
- **PANTHER:** PANdemic preparedness plaTform for Health and Emerging infectious Response
- **PD:** pharmacodynamic
- **PHEIC:** public health emergency of international concern
- **PK:** pharmacokinetic
- **POC**: proof-of-concept
- **Preclinical Compounds with only preclinical data and no clinical data designated as:**
  - **Hit –** high-throughput or compound library screening hit, initial antiviral activity requiring significant optimization. Limited or no *in vitro* data available supporting antiviral mechanism of action (MOA).
  - **Early Lead –** limited Structure-Activity Relationship (SAR), antiviral activity associated with MOA, may have limited *in vitro/in vivo* pharmacokinetic data reported.
  - **Late Lead –** potency consistent with candidate quality for the specific MOA, more extensive *in vitro* characterization (e.g., ADME profile, activity against clinically relevant virus strains/isolates), *in vivo* PK and/or animal efficacy model data reported.
  - **Potential Candidate –** *in vivo* efficacy and safety dataset consistent with preparation for FDA IND (or similar) submission; compound has been reported by developer as a pipeline clinical candidate and/or in IND (or similar) enabling studies.
  - **Archived –** progress on the compound has been stopped (timeframe stopped, >5 years); antiviral evidence is only computational; previously optimized drug from another antiviral/other indication that only has weak activity.

Glossary of Terms (cont'd)

- **Preclinical Exploratory** are Investigational ("unapproved") and Approved antivirals exploring antiviral activity against a different virus from the Investigational/Approved antiviral indication, including:
  - **Approved Antiviral-Indication Expansion** – antiviral approved for one or more viral disease indications.
  - **Investigational Antiviral-Indication Expansion** – antiviral in clinical development, not yet approved.
- **'Promising' Compound**: clinical compound that aligns with 100 Days Mission goals and/or has been registered and approved for established viral diseases
- **RCT:** randomized controlled trial
- **SAM:** S-adenosylmethionine
- **USAMRIID:** U.S. Army Medical Research Institute of Infectious Diseases
- **VACV:** vaccinia virus
- **VARV:** variola virus

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