Nigerian Journal of Pharmaceutical and Biomedical Research

Vol. 10 Issue.2 August, 2026. p-ISSN: 2579-1419 e-ISSN: 2814-1423

MPOX VIRUS (MONKEYPOX): MOLECULAR EVOLUTION, HOST ADAPTATION, IMMUNE EVASION, DIAGNOSTIC INNOVATIONS, AND FUTURE THERAPEUTIC STRATEGIES

Tamara Amer Taha[1], Khloud Adnan AL-Lehaibe[2]

[1]College of Basic Education, University of Diyala, Iraq
[2]Department of Biology, Faculty of Education for Pure Sciences, University of Diyala, Diyala, Iraq
ORCID: 0000-0003-4332-7356, https://orcid.org/0000-0003-1600-4391[2]
Corresponding author Email: basicsci28@uodiyala.edu.iq,basicsci3@uodiyala.edu.iq[2]
DOI 10.5281/zenodo.24208516

ABSTRACT

This re-emergence, combined with multi-country outbreaks that began in 2022 and spread via two Clade I strains (specifically Clade Ib), has elevated MPXV from an endemic zoonosis to a global public health emergency of international concern (PHEIC). This review provides an overview of the molecular structure, genome biology, evolution and immunopathogenesis of MPXV. We outline the role played by APOBEC3 in host adaptation and the speed of mutation accumulation within Clade IIb, and the structural rearrangements and gene loss that contribute to clinical morbidity and immune escape in Clade I.

Moreover, we summarize how MPXV mechanistically targets innate and adaptive immune parameters, including the ability to inhibit cGAS-STING, NF-κB, and type I interferon signaling pathways, and disrupt CD4+/CD8+ T-cell reactions. On the translational front, we discuss progress made on next-generation diagnostics, such as multiplex isothermal amplification (CRISPR-Cas coupled assays and LAMP), digital droplet PCR (ddPCR), and in-field nanopore sequencing, in comparison with current clinical benchmarks. We discuss extensively the Durability and Immunological coverage of existing Modified Vaccinia Ankara (MVA-BN) and LC16m8 vaccines against emergent lineages, as well as the clinical profile of direct-acting antivirals (Tecovirimat, Brincidofovir) and novel druggable targets. Finally, we define a One Health genomic surveillance architecture to monitor the rodent-human interface, to track antigenic shifts and to prevent new pandemic threats.

Keywords: Mpox virus; Orthopoxvirus; Molecular evolution; APOBEC3 cytidine deaminase; Immune evasion; CRISPR-Cas diagnostics; Tecovirimat; MVA-BN vaccine; One Health surveillance.

1. INTRODUCTION

The poxvirus Mpox (MPXV), one of the most well-known members of the Orthopoxvirus genus in Poxviridae, was thought to be a rare and understated zoonosis confined to the forested basins of Central (Clade I) and West (Clade II) Africa until recent years (Adler et al., 2022).MPXV was first recorded in cynomolgus monkeys in Copenhagen in 1958, and clinically confirmed in humans in the Democratic Republic of the Congo (DRC) in 1970 and has been seen to be maintained by both self-sustaining chains of human-to-human transmission as well as

occasional spillover into humans from forest-living rodents and squirrels (Americo et al., 2023).

This historical paradigm however, fell apart in May 2022, with an unprecedented multi-country outbreak occurring in non-endemic countries in Europe, the Americas and other continents. The transmission was primarily via close and sustained intimate networks, and resulted in the successive declarations of the World Health Organisation (WHO) as Public Health Emergency of International Concern (PHEIC) (Antinori et al., 2024). A new rapidly expanding sublineage of MPXV (Bunge et al., 2022), that arose and spread within Central Africa, further demonstrates the evolutionary plasticity of MPXV.

While mpox research has made significant strides, there is a critical need to understand the implications of discoveries related to viral evolution and host–virus interaction for mpox diagnosis, prevention, treatment and surveillance. (Happi et al., 2022) The recent report of clade Ib further supports the need for a shared framework to study these interrelated domains. Recent reviews of the evolution, pathogenesis, diagnostics, vaccines, and therapeutics of MPXV are reviewed here, and the present review takes a holistic view, linking molecular mechanisms with clinical and public-health outcomes. One of its unique strengths lies in its focus on the links between genomic variation, host adaptation, immune evasion and the efficacy and/or failures of existing and developing countermeasures (Bunge et al., 2022). In line with this, the current review aims to summarise the current knowledge on MPXV biology and evolution, understand the mechanisms of pathogenesis and immune escape, review the diagnostic technologies currently available and the preventive and therapeutic measures available, and highlight the areas of research priority that are relevant to the context of One Health preparedness. Ongoing results are separated from experimental results and suggested future uses throughout. (Antinori et al., 2024)

2. MATERIALS AND METHODS

This narrative review covers the molecular architecture, genome Organisation, evolution, host interaction, immune evasion, clinical features, epidemiology, diagnostic progress, vaccines, antiviral therapies, and One Health surveillance of mpox virus (MPXV). The manuscript used publications such as original research articles, review articles, a reference textbook chapter, and World Health Organisation guidance as source material.

When creating the list of sources, the ones that covered one or more of the main themes of the review were deemed relevant. Related orthopoxviruses provided background information as evidence to support the interpretation of viral replication, host responses and development of countermeasures. This evidence should be separated from direct evidence found in MPXV. The review did not include publications that did not relate to these fields.

The literature was qualitatively compiled and categorized into thematic sections. A discussion was conducted that included molecular and experimental information as well as clinical and public health evidence, and demonstrated how they relate to the understanding of MPXV and the development of diagnostic, preventive, and therapeutic strategies. There was no quantitative pooling or meta-analysis.

This review did not record a reproducible database search strategy, pre-defined publication date range, final search date or formal screening log. This means that the

number of retrievals, the number of exclusions at the screening stage or database-specific Boolean searches cannot be accurately reported to the user. There is no formal risk-of-bias assessment. The review is therefore not intended to be a comprehensive systematic review and should not be interpreted as such, due to the possibility of selection bias and lack of full representation of the available evidence.

3. LITERATURE REVIEW

3.1. Taxonomy and Molecular Architecture of Mpox Virus

MPXV is a member of the Chordopoxvirinae subfamily of poxviruses that infect vertebrates. MPXV is not a spherical, icosahedral or enveloped helical virus, but a large, very complex brick-shaped virion that measures about 200–250 nm in width and 250–350 nm in length. (Happi et al., 2022). It possesses a very complex molecular Organisation, with four main structural compartments:

- **Biconcave Nucleoprotein Core:** The 197-kbp linear double-stranded DNA genome is enclosed in a biconcave Nucleoprotein Core that contains all the structural proteins (e.g., A10L and A4L) and a complete viral DNA-dependent RNA polymerase multisubunit complex, capping enzymes, and poly(A) polymerase.
- **Lateral Bodies**: Amorphous elliptical bodies containing protein which are located within the core concavities. These bodies serve as molecular storage depots that, upon fusion of their membranes with the host, liberate virulence and immunomodulatory effector proteins that directly enter the host cytoplasm and are present before the host's first antiviral response is activated.
- **The Inner Core Membrane and Palisade Layer**: A proteinaceous lattice that is very dense and surrounds the nucleoprotein core, which is very tightly bound and resistant to environmental desiccation and to detergent disruption.
- **The infection cycle generates two morphologically and antigenically distinct functional forms:** (1) Intracellular Mature Virus (IMV) has a single lipid envelope and is highly stable in the environment, and is responsible for external host-to-host transmissibility; and (2) Extracellular Enveloped Virus (EEV) is enclosed in an additional lipid membrane, derived from the host cells, which contains specialized glycoproteins (e.g., A33R, B5R) responsible for rapid systemic spread within the host, while in the process, the virion is protected from neutralizing humoral antibodies.(Bunge et al., 2022)

3.2 Genome Organisation and Viral Evolution

3.1 Genomic Topography and Open Reading Frames

MPXV genome is a large linear double-stranded DNA molecule that contains 180 to 190 annotated open reading frames (ORFs) of about 197 kbp. The whole genome is divided into three functional domains: one is a highly conserved central domain (~ 100 kbp), and the other two are terminal regions (differential) containing identical Inverted Terminal Repeats (ITRs) with covalently closed hairpin termini. (Happi et al.,2022)

The central core contains essential housekeeping genes for DNA replication (E9L polymerase, A20R processivity factor), transcription, virion morphogenesis

and structural scaffolding. On the other hand, the variable terminal regions of the periphery code for virulence factors, host-range determinants and cytokine decoys. There are regions flanking these that have a high degree of genomic fluidity with direct involvement in lineage-specific virulence and species tropism through deletions, tandem duplications and non-homologous recombination events. (Antinori et al., 2024)

3.2 Evolutionary Drivers: APOBEC3 Cytidine Deamination and Gene Accordions

Historically, poxviruses have had low baseline mutation rates (~ 10^-6 to 10^-5 substitutions per site per year) from the 3'-to-5' exonuclease proofreading activity of the E9L DNA polymerase. Comparable studies with the 2022-2026 Clade IIb outbreak and newly identified strains of Clade Ib, however, suggested that the evolutionary rate of the Clade IIb strains is 6- to 12-fold faster than expected phylogenetic neutral drift. (Kumar et al., 2023).

Host apolipoprotein B mRNA editing catalytic polypeptide-like 3 (APOBEC3) enzymes are the major contributors to this microevolution. The host innate cytidine deaminases cause specific nucleotide substitutions, most frequently 5'-TC->TT and 5'-GA->AA. In addition to APOBEC3 mutational pressure, poxviruses take advantage of 'genomic accordions' which involve rapid duplication of adaptive sites under host immune pressure, nucleotide fine-tuning, and later contraction to optimise fitness.(Hatch et al., 2025)

3.3 Replication Cycle and Host Cell Interaction

The exclusive property of MPXV biology is its strictly cytoplasmic life cycle (no involvement of the host cell nuclear transcription machinery). Replication is a carefully orchestrated process with steps:

•**Attachment and Entry:** IMV and EEV have different means of entry. IMV binds to cell surface glycosaminoglycans (chondroitin sulfate and heparan sulfate) and uses apoptotic mimicry through the exposure of phosphatidylserine to trigger macropinocytosis. Upon contact, EEV releases its fragile outer membrane, allowing the IMV core to fuse directly with the plasma membrane or the endosomal membrane with the help of the multiprotein entry-fusion complex (EFC) that consists of 11 conserved viral proteins. (Isidro et al., 2022).

Early Transcription and Primary Uncoating: As soon as the virion enters the cytoplasm, the pre-packaged viral RNA polymerase transcribes early genes for components of the viral replicase, transactivators and immune suppressor proteins. The viral genome enters special replication territories after primary uncoating.

Viral DNA Replication: Viral DNA synthesis occurs in specific perinuclear areas that are bounded by the endoplasmic reticulum (ER) and referred to as viral factories. The template strand is self-primed, and the template is displaced in a self-priming rolling-hairpin manner, resulting in the formation of high molecular weight head-to-head concatemers. (Kaler et al., 2022).

Morphogenesis and Virion Assembly: Intermediate and late viral genes promote the assembly of ER-Golgi intermediate compartment (ERGIC) derived crescent-shaped membranes. Concatemeric DNA is separated into monomeric units and is

enveloped into spherical immature virions (IVs) that are structurally modified to form stable brick-shaped IMVs

Egress and Actin Rocketry: A small proportion of IMVs are transported to the trans Golgi network, where they become intracellular enveloped virions (IEVs), a modification that involves a further double membrane. This wrapping is mediated by the viral F13L protein (VP37). The outermost membrane fuses with the host plasma membrane, and the cell-associated enveloped virus (CEV) is displayed. The viral proteins recruit host cellular N-WASP and Arp2/3 complexes and polymerise dense actin projections ('actin rockets') which project the progeny virion rapidly into the neighbouring host cells. (Isidro et al., 2022)

3.4 Pathogenesis and Clinical Manifestations

After inoculation through the skin or penetration into the mucous membranes, MPXV replicates locally in keratinocytes, dermal dendritic cells and tissue macrophages. The infection spreads quickly into the regional lymph nodes to induce primary viremia and then to reticuloendothelial organs (liver and spleen) and finally to secondary viremia with seeding of the mucocutaneous and visceral organs. (Isidro et al., 2022)

The incubation period ranges from 5 - 21 days (mean 6-13 days). Clinical illness can be divided into two well-defined, sequential periods: (1) the Prodromal Phase (Days 1–4): fever, rigors, cephalalgia, severe myalgia, back pain, profound asthenia and marked lymphadenopathy (submandibular, cervical and inguinal). The most distinguishing clinical finding of Mpox from smallpox and varicella is marked lymphadenopathy. During (2) the Eruptive Phase, which features well-circumscribed, deep-seated lesions, the lesions move forward in synchrony from macules, through papules, vesicles, umbilicated pustules, and desquamating crusts.(Kaler et al., 2022).

This classic style presentation was changed by the transmission patterns of the 2022-2026 period. Clinical disease is often characterised by focal lesions of the mouth, anus, and vulva; proctitis, urethritis and extensive lesions of the mucous membrane with or without marked systemic fever. Severe complications include secondary bacterial superinfections, keratitis and permanent corneal scarring, bronchopneumonia, and demyelinating post-viral encephalomyelitis, the latter more often seen in the immunocompromised host, who may have an unsuppressed HIV infection(Kumar et al., 2023)**.**

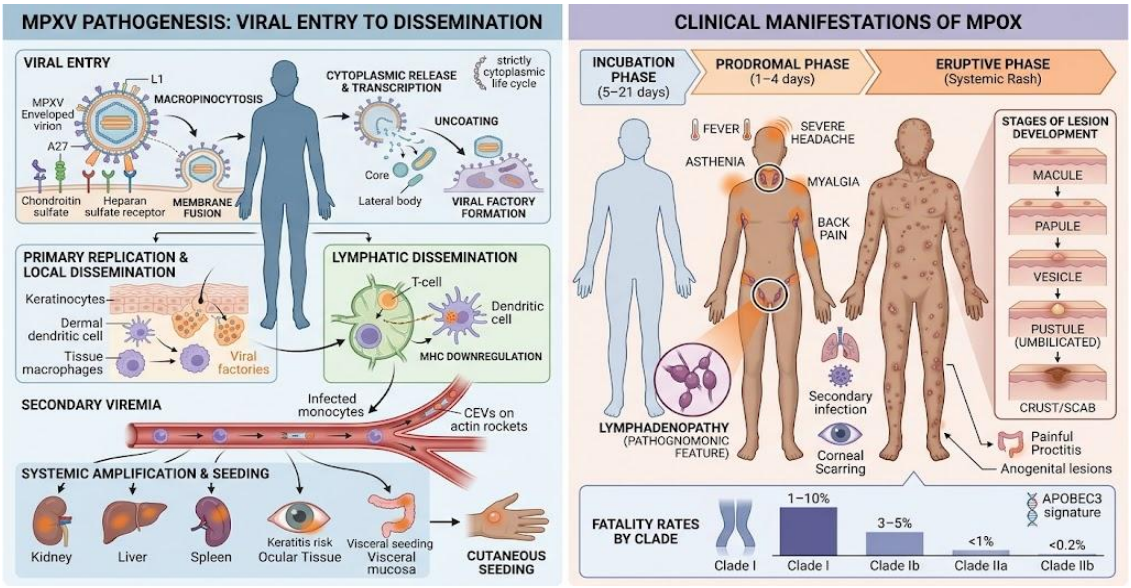

Figure 1: Mechanistic Pathogenesis, Lymphatic Dissemination, and Staged Clinical Progression of Mpox (Lapa et al., 2022)

This figure gives a comprehensive overview of Mpox pathogenesis and clinical presentations. The left shows the viral steps leading from entry (attachment factors and cell entry mechanisms such as micropinocytosis and membrane fusion) to the initial replication and dissemination to the tissue cells. It demonstrates the need for lymphatic dissemination and the ability of the virus to down-regulate MHC and evade the immune system, resulting in a strong secondary viremia. Last, it visualises the systemic seeding, where infected monocytes and virions spread to important organs and cause eye complications, mucosal lesions in the viscera and eventually, seeding in the skin (Happi et al., 2022).

On the right, the clinical history and symptoms are shown, with the incubation period, prodromal period (high fever and back pain), and eruptive period, followed by a generalised systemic rash. It emphasises the distinctiveness of enlarged lymph nodes. A detailed inset box visualises the morphological changes in lesions that are synchronous and show the macule, papule, vesicle, umbilicated pustule and scab, with skin cross-sections in each. Finally, it presents the clade-specific lethality, as well as a comparison between historic Clade I strains and recent emergent Clade IIb and Ib strains, which show a lower mortality rate but a different hypermutation signature (Moss, 2023)

3.5 Immune Response and Immune Escape Mechanisms

MPXV dedicates about one-third of the whole genome to the ability to subvert various aspects of innate and adaptive immune defences, and MPXV possesses a very sophisticated molecular armoury.(Mitjà et al., 2023).

cGAS-STING and Cytosolic DNA Sensing Inhibition: Host cyclic GMP-AMP synthase (cGAS) senses viral dsDNA in the cytosol and generates 2'3'-cGAMP to activate STING and promote type I IFNs. MPXV has viral poxins (viral nucleases encoded by B16/B15R), which enzymatically cleave 2'3'-cGAMP, thereby shutting down the STING activation cascade prior to signalling.

Blockade of Type I Interferon Signalling: MPXV produces soluble decoy

Interferon α/β receptors which bind to cytokines in the extracellular microenvironment. In the cytoplasm, the viral E3L ortholog binds and sequesters the viral dsDNA, preventing it from being recognised by PKR and 2'-5'-oligoadenylate synthetase (OAS). The viral K3L protein is a molecular decoy substrate for phosphorylated PKR and inhibits eIF-2α translation initiation (Americo et al., 2023).

NF-κB Suppression: Multiple viral ankyrin-repeat proteins (e.g., orthologs of B14R and CP77) physically interact with host Cullin-RING ubiquitin ligase complexes to block phosphorylation and proteasomal degradation of IκBα, resulting in NF-κB suppression. This way, NF-κB stays in the cytoplasm and cannot promote pro-inflammatory transcription.

Apoptosis Inhibition and Host Survival: MPXV contains viral Bcl-2 structural homologs that are anchored to the mitochondrial outer membrane, bind pro-apoptotic Bax/Bak proteins and block mitochondrial outer membrane permeabilisation (MOMP), thereby allowing host survival for viral factory assembly (Gillard et al., 2026).

Impairment of Antigen Presentation: Active downregulation of MHC-I and MHC-II expression (both classes I and II) at the cell surface by viral proteins leads to a failure in the activation of CD8+ T-lymphocytes, which are required for killing virus-infected cells, and failure in the maturation of neutralising antibody responses by CD4+ T-cells, which are required to orchestrate antibody maturation.(Kaler et al., 2022).

3.6 Epidemiology and Transmission Dynamics

Previous to the current study, MPXV was known to be present in two distinct geographical and clinically distinct clades, the Congo Basin clade (MPXV Clade I) and the West African clade (MPXV Clade II). A standardised nomenclature was adopted in 2022, with Clade I (comprising sublineages Ia and Ib) and Clade II (sublineages IIa and IIb). Clade Ia (case fatality rate (CFR) up to 10%, mainly the case-fatality and household amplification phase) and Clade IIb (CFR <0.2%, sustained transmission in close-contact intimate networks) were the two main drivers of the global pandemic of 2022 (Moss, 2023)

Clade Ib is a significant shift as it shows evidence of high human-to-human transmissibility both through close contact and through household contacts and is estimated to have a 3%-5% case fatality rate. High regional mobility, dense population focus, cross-border trade and the presence of large groups of unimmunized people (who were not vaccinated when universal smallpox vaccination was discontinued about 1980) have increased

the vulnerability to a pandemic (Nishiura et al., 2025).

3.8 Molecular Diagnosis and Emerging Technologies

Effective outbreak interruption relies on rapid, sensitive, and clade-discriminative diagnostics. Real-time quantitative polymerase chain reaction (qPCR) with dual-target hydrolysis probes, which are the same as the current reference method, is the method used. Highly conserved regions, including the G2R gene (a gene coding for a tumour necrosis factor receptor homologue), the F3L gene or the C18L gene, are recommended and are typically accompanied by a pan-orthopoxvirus assay

to confirm genus membership (Antinori et al., 2024).

Centralized qPCR requires advanced thermocycling equipment, cold chain reagents, as well as trained staff, making it impractical to use in resource-limited endemic settings. To address such challenges, novel platforms for diagnostics have emerged:

Loop-Mediated Isothermal Amplification (LAMP): LAMP amplifies DNA by way of a strand-displacement reaction at constant temperatures (60-65°C) and produces results in 20-40 minutes that are visible with a colourimetric change in pH or with a portable fluorometer. (Adler et al., 2022; Happi et al., 2022)

Platforms based on Cas12a (DETECTR) and Cas13a (SHERLOCK) leverage guide RNAs (crRNAs) that are programmable for single-nucleotide specificity with target sequences unique to quenched fluorescent or lateral-flow reporters, allowing for visual detection of target binding within 45 minutes. MPXV. Cas12a cleaves quenched fluorescent or lateral-flow reporter molecules, allowing visual detection of target binding within 45 minutes. Cas12a cleaves reporters of any kind that are droplet-based. Digital PCR (ddPCR) quantifies viremia without a standard reference curve by breaking down a clinical sample into 20,000 distinct droplets of water in oil, to measure low-level viremia and subclinical mucosal carriage, for the first time, with unprecedented sensitivity.(Rao et al., 2023).

Field-Deployable Nanopore Sequencing: Pocket-sized sequencing instruments such as Oxford Nanopore MinION enable full genome sequencing of samples in under 12 hours to provide actionable phylogenomic and clade-differentiation data, directly in field clinics.

3.9 Vaccines and Preventive Strategies

Smallpox vaccines provide significant cross-protection against MPXV, due to the high degree of similarity between the two. First-generation calf-lymph vaccines (Dryvax) and second-generation cell-culture vaccines (ACAM2000) use a replication-competent vaccinia virus and are contraindicated in immunocompromised hosts and have significant risks of myopericarditis, progressive vaccinia and accidental inoculation (Reynolds et al., 2026).

The international cornerstone today is MVA-BN (Modified Vaccinia Ankara - Bavarian Nordic; marketed as JYNNEOS, IMVANEX or IMVAMUNE). MVA-BN is a third-generation, highly attenuated, replication-incompetent vector that has been passaged more than 570 times in primary chick embryo fibroblasts, leading to extensive genomic deletions which do not allow the vector to replicate in mammalian cells. MVA-BN has an outstanding safety profile when given subcutaneously or by dose-sparing intradermal injection, even in people living with HIV. It elicits potent neutralisation antibody responses to both IMV antigens (L1R and A27L) and EEV antigens (A33R and B5R) as well as robust T cell responses. (Moss,2023).

Fourth-generation vaccines are versions of the original vaccine that have been altered to stop the bacteria from replicating significantly, such as LC16m8, which is an attenuated (weakened) form of the Lister vaccine developed in Japan that is only minimally replicating. The B5R envelope glycoprotein is not expressed in LC16m8, which significantly lowers its neurovirulence while keeping its protective

cellular and humoral immune response in single-dose regimens. Aside from viral vectors, the next generation of lipid nanoparticle (LNP)-encapsulated mRNA vaccines with multivalent surface antigen cocktails (e.g., A29L, M1R, A35R, and B6R) is moving forward in clinical pipelines, providing a more rapid and scalable manufacturing process with more targeted immunogenicity. (Titanji et al., 2022)

3.10 Antiviral Therapy and Drug Development

Pharmacological treatments for serious mpox disease are based on direct-against-virus medications that were stockpiled for weaponised smallpox:

Tecovirimat (ST-246 / TPOXX): The frontline therapeutic agent. Tecovirimat binds to the conserved portion of the viral F13L gene product (VP37 envelope wrapping protein), which blocks IMV binding to host trans-Golgi membranes and Rab9 GTPase. This will stop the formation of EEVs and actin rocketry, keeping the virus in the primary infected cell. Tecovirimat is safe and well tolerated and is available both orally and intravenously. One of its drawbacks, however, is that its genetic resistance is low, as evidenced by the presence of several fixed SNPs in F13L that provide strong resistance, such as F315L and A290V. (Mitjà et al., 2023).

Brincidofovir (CMX001 / Tembexa): orally bioavailable, lipid-conjugated prodrug of the acyclic nucleotide phosphonate cidofovir. Due to the lipid ester moiety, the drug is better taken up by the cells and avoids renal organic anion transporters, thus preventing the severe nephrotoxicity associated with cidofovir parenteral administration. Cidofovir diphosphate is released following intracellular cleavage, and selectively inhibits viral DNA polymerase (E9L) to cause chain termination.

NIOCH-14 and Investigational Molecules: NIOCH-14 is a chemically modified tricyclic dicarboxylic acid derivative, which is an analogue of Tecovirimat with improved chemical stability, bioavailability, and activity against some mutants of F13L. New high-throughput screening efforts centre on host-directed therapeutics, including selective inhibitors of the retrograde trafficking pathway (Retro-2), and allosteric inhibitors of viral DNA processivity factor A20R. (Titanji et al., 2022).

3.11 Genomic Surveillance and One Health Preparedness

To prevent the threat of the Mpox pandemic, we need to shift from reacting to containment to a One Health strategy bringing together human, veterinary, and environmental epidemiology and moving forward.

- To determine ecological tipping points that could trigger viral spillovers, it is essential to elucidate the exact enzootic reservoir cycle, including tracking of the arboreal and terrestrial rodents (Funisciurus rope squirrels, Heliosciurus sun squirrels and Cricetomys gambianus pouch rats).

-Wastewater-Based Epidemiology (WBE): Monitoring of municipal and community wastewater is highly sensitive for detecting community-level MPXV DNA shedding days before the first clinical cases are reported, providing an early warning system "outbreak radar".

Real-Time Genomic Consortia: Continuous sequencing and timely open-access depositing in global sequencing repositories (such as GISAID, Nextstrain) are essential to identify antiviral resistance mutations of F13L/E9L, track APOBEC3

mutational footprints and monitor potential recombination events among co-circulating orthopoxviruses. (Vaughan et al., 2026).

3.12 Current Challenges and Future Perspectives

There are still some huge gaps in our systems, despite scientific advances. International countermeasures are highly inequitable, with endemic nations with limited resources in the global South having access to limited supplies of MVA-BN vaccines and Tecovirimat, despite having the highest disease burden. (Gillard et al., 2026). Important knowledge gaps still exist regarding the persistence of mucosal immunity after vaccination, subclinical transmission rates, and chronic sequelae in patients with high immunosuppression. Development of next-generation pan-orthopoxvirus antivirals with high genetic resistance barriers, scalable multi-antigen mRNA vaccines that elicit sterilising mucosal immunity and low-cost, instrument-free point-of-care diagnostics that are readily accessible in primary health care facilities around the world are priorities for future research. (World Health Organization, 2024).

4. CONCLUSION

The evolution of Mpox virus from an obscure zoonosis into an endemic and pandemic threat underscores the relentless fluidity of emerging viral pathogens in an interconnected world. By bridging fundamental viral biochemistry with innovative translational medicine, the global scientific community can mount an enduring, coordinated defence against MPXV, ensuring preparedness, therapeutic security, and global health equity.

Table 1: Taxonomic and Biological Classification of Mpox Virus

Metric / Parameter	Biological Specification	Clinical / Virological Significance
Order	Chitovirales	Defines large, structurally complex double-stranded DNA viruses.
Family	Poxviridae	Distinguished by brick-shaped architecture and cytoplasmic life cycle.
Subfamily	Chordopoxvirinae	Encompasses poxviruses naturally infecting vertebrate hosts.
Genus	Orthopoxvirus	Exhibits broad antigenic and serological cross-reactivity.
Species	Mpox virus (MPXV)	Re-emergent zoonosis capable of sustained human-to-human spread.
Genome Topography	Linear dsDNA (~197 kbp)	Terminal inverted repeats; non-nuclear transcription and replication.
Physical Dimensions	200 × 250 × 350 nm	Exceptionally large virion; detectable under high-end transmission EM.

Table 2: Genome Architecture and Critical Functional Open Reading Frames (ORFs)

Functional Category	Representative Genes	Primary Biochemical & Cellular Function
DNA Replication Machinery	E9L, A20R, D4R, D5R	Encodes catalytic DNA polymerase, processivity factor, uracil-DNA glycosylase, and primase-helicase complex.
Innate Immune Evasion	E3L, K3L, B15R, C12L	Inhibits PKR phosphorylation, blocks eIF-2α translational arrest, sequesters IL-1β, and neutralises type I IFN cascades.
Virion Scaffolding & Structure	A10L, A4L, L4R, D13R	Core structural proteins, palisade assembly, and rifampicin-sensitive scaffold formation for immature virions.
Envelope & Attachment	A27L, L1R, A33R, B5R	Mediates GAG binding, membrane fusion complex (EFC) function, and EEV outer envelope stabilisation.
Envelopment & Cell Egress	F13L (VP37)	Coordinates trans-Golgi wrapping of IMVs into IEVs; primary target of Tecovirimat.
Genomic Termini & Plasticity	ITR Elements, C18L, F3L	Covalently closed hairpin telomeres critical for rolling-hairpin replication and host-adaptation accordions.

Table 3: Comparative Evaluation of Molecular Diagnostic Methodologies

Platform	Target Analyte	Turnaround	Analytical Sensitivity	Field Utility
Real-Time qPCR	G2R / F3L / C18L	1–3 hours	> 99% (Gold Standard)	Low (Central laboratory)
Isothermal LAMP	Terminal ITR loci	25–40 min	90–95% (High)	High (Point-of-care)
CRISPR-Cas12a/13a	Clade-specific SNPs	30–60 min	> 98% (Near gold std)	Very High (Lateral flow)
Droplet Digital PCR	Low copy viral DNA	3–5 hours	Absolute quantification	Low (Specialised centres)
Nanopore MinION	Whole viral genome	6–12 hours	High sequence depth	High (Mobile outbreak teams)

virus finallyTable 4: Current and Investigational Antiviral Therapeutics

Therapeutic Agent	Molecular Target	Mechanism of Action	Administration	Resistance Barrier
Tecovirimat (TPOXX)	F13L (VP37)	Inhibits trans-Golgi wrapping & EEV egress	Oral / Intravenous	Low (Single SNPs, e.g. F315L)
Brincidofovir (CMX001)	E9L (Polymerase)	Lipid prodrug halting viral DNA elongation	Oral	High (Preserves renal profile)
Cidofovir	E9L (Polymerase)	Nucleotide analogue terminating DNA chains	Intravenous	High (Severe nephrotoxicity)
NIOCH-14	VP37 homologue	Structural analogue inhibiting egress	Oral	Moderate (Active vs select SNPs)
Retro-2	Host retrograde pathway	Blocks intracellular retrograde trafficking	Investigational	High (Host-directed therapy)

Table 5: Comparative Landscape of Poxvirus Vaccine Platforms

Vaccine	Vaccine Type	Gen.	Replication	Target Clinical Populations
MVA-BN (JYNNEOS)	Modified Vaccinia Ankara	3rd	Non-replicating	General population, PLWH, pre-exposure
LC16m8	Lister-derived attenuated	4th	Minimally replicating	Broad population; single-dose protocol
ACAM2000	Live clonal vaccinia	2nd	Fully competent	Healthy military adults (Myopericarditis risk)
Multivalent mRNA	LNP-encapsulated mRNA	Next	Non-replicating	Clinical pipeline; targeted IMV/EEV antigens

2. Scientific Figures and Legends

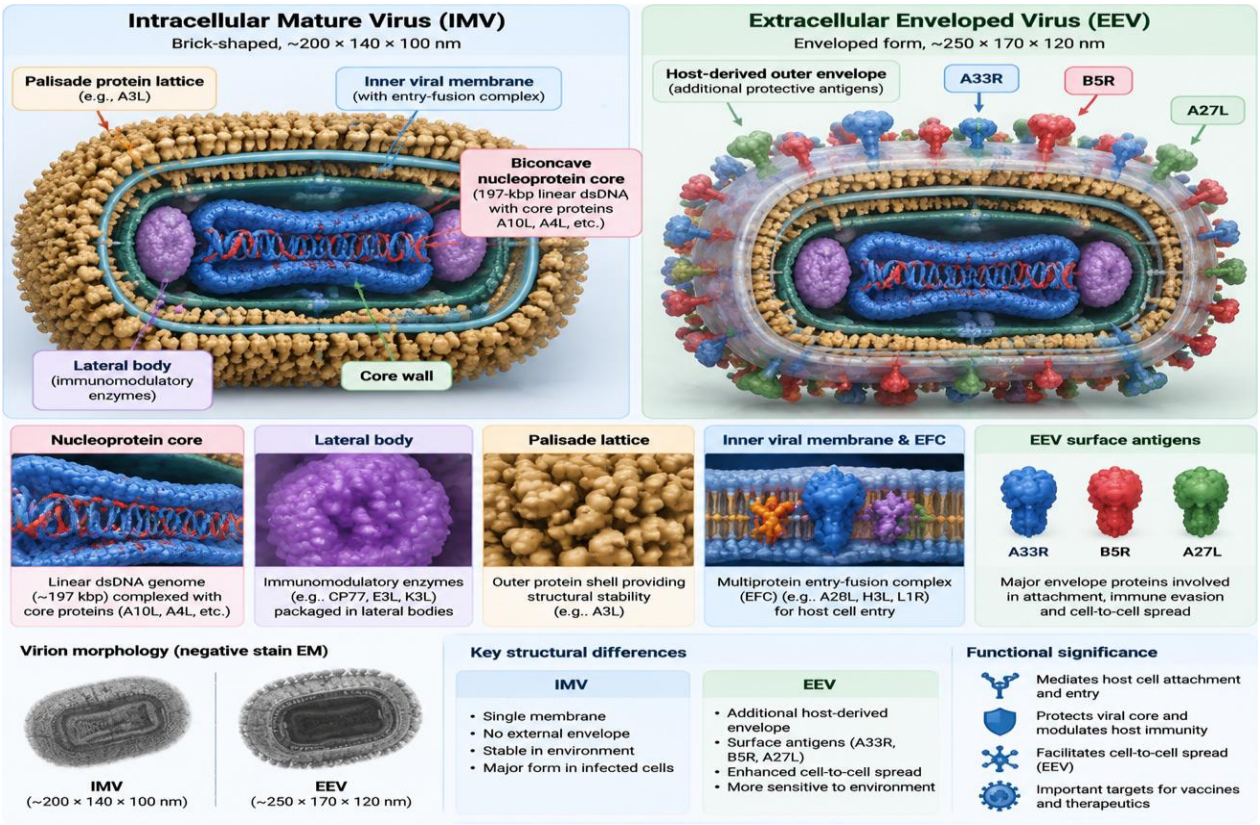

Figure 2: Architecture of the Mpox Virion.

Conceptual cutaway illustrating the structural morphology of both Intracellular Mature Virus (IMV) and Extracellular Enveloped Virus (EEV). Key elements highlighted include the central biconcave nucleoprotein core enclosing the 197-kbp linear dsDNA genome complexed with core proteins (A10L, A4L), flanking lateral bodies carrying immunomodulatory enzymes, the palisade protein lattice, and the inner viral membrane studded with the multiprotein entry-fusion complex (EFC). The outer host-derived envelope of the EEV form displays distinct protective antigens, including A33R, B5R, and A27L.(Moss,2023).

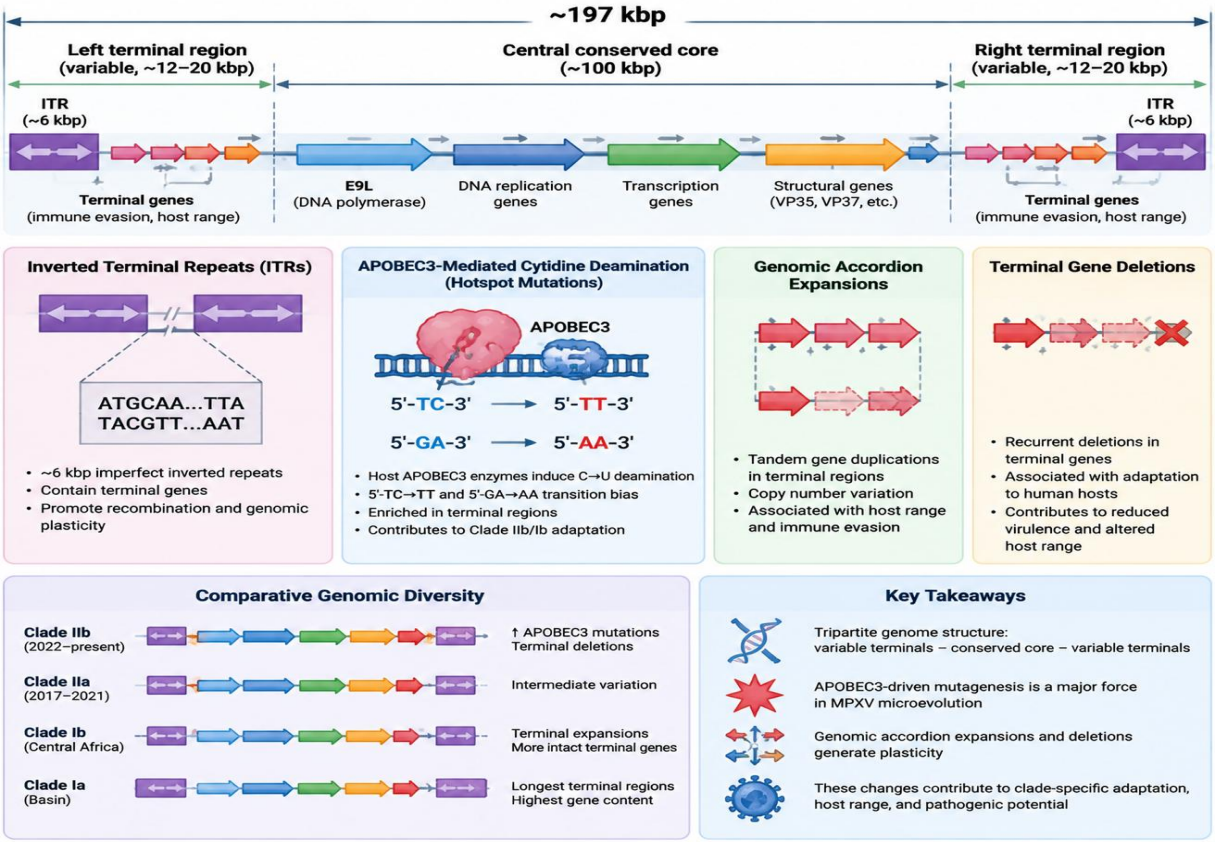

Figure 3: Linear Topography and Microevolutionary Plasticity of the MPXV Genome. Genomic schematic illustrating the tripartite organization of the 197-kbp double-stranded DNA genome. Depicted is the central conserved core (~100 kbp) encoding replication (E9L) and transcription apparatus, flanked by hypervariable terminal regions and Inverted Terminal Repeats (ITRs). Annotations illustrate hotspot loci of host APOBEC3-mediated cytidine deamination (characterized by 5'-TC->TT and 5'-GA->AA transition bias) driving Clade IIb/Ib adaptation, alongside genomic accordion expansions and terminal gene deletions. (Hatch et al., 2025)

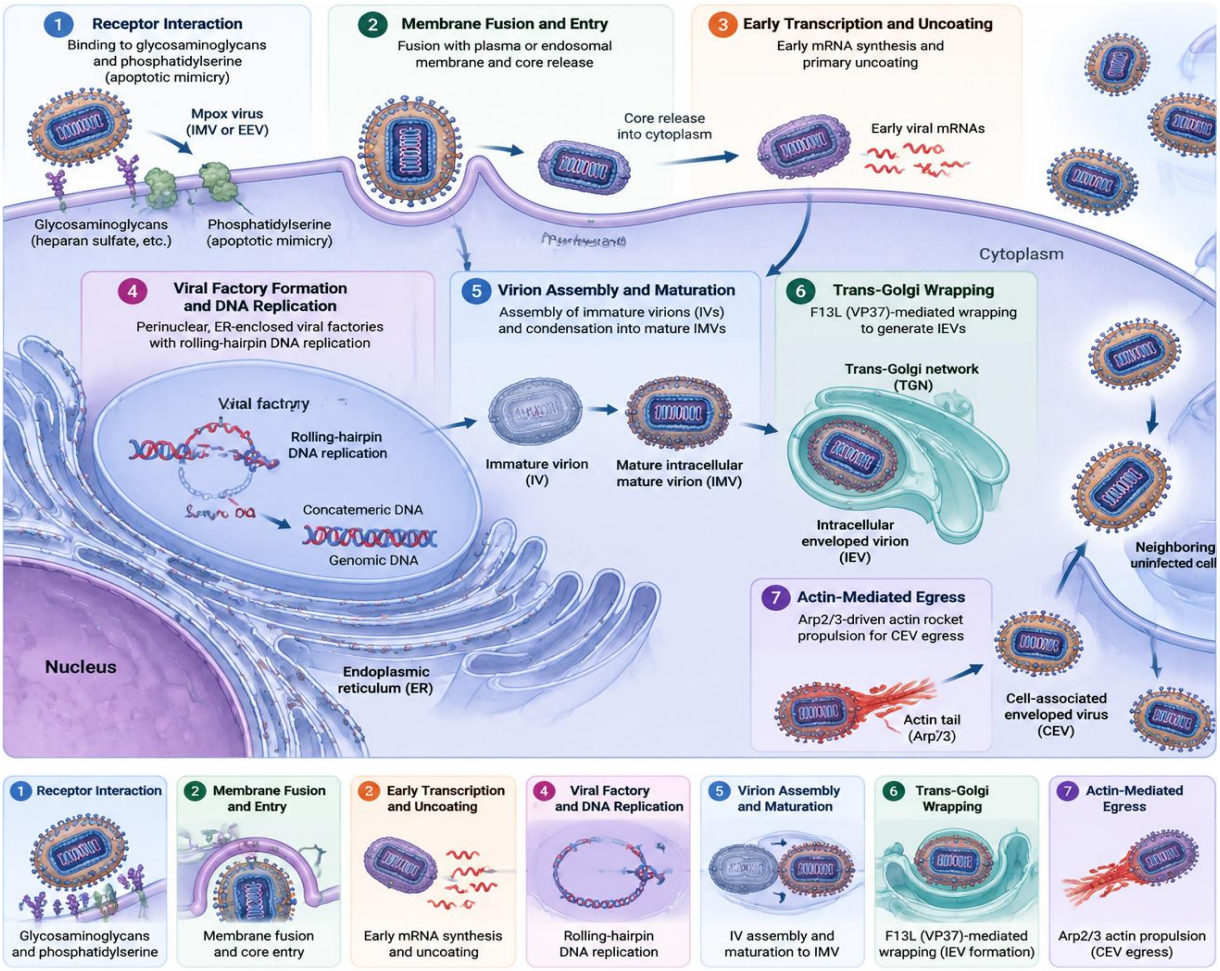

Figure 4: Comprehensive Spatiotemporal Cytoplasmic Replication Cycle of Mpox Virus. Schematic detailing the life cycle within the host cell: (1) Receptor interaction via glycosaminoglycans and phosphatidylserine apoptotic mimicry; (2) Membrane fusion and core entry into cytoplasm; (3) Early transcription and primary uncoating; (4) Assembly of perinuclear, ER-enclosed viral factories for rolling-hairpin DNA replication; (5) Immature virion (IV) assembly and condensation into mature IMVs; (6) Trans-Golgi wrapping by the F13L (VP37) complex to generate IEVs; and (7) Arp2/3-directed actin rocket propulsion driving cell-associated enveloped virus (CEV) egress into neighboring uninfected cells.(Hatch et al., 2025)

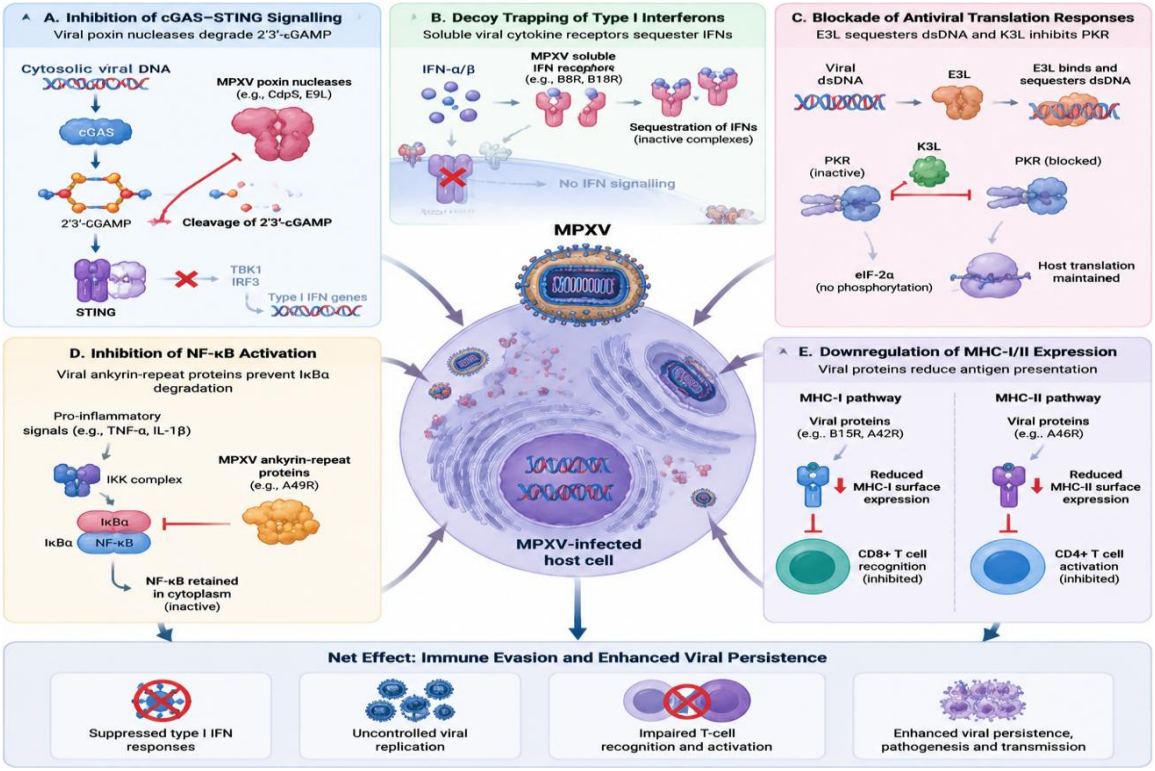

Figure 5: Molecular Cascades of MPXV-Mediated Innate and Adaptive Immune Evasion.

Intracellular signalling diagram illustrating the multi-tiered mechanisms deployed by MPXV virulence proteins: (A) Cleavage of 2'3'-cGAMP by viral poxin nucleases to neutralize the cGAS-STING cytosolic DNA-sensing axis; (B) Decoy trapping of extracellular type I IFNs by soluble cytokine receptors; (C) Sequestration of dsDNA by E3L and competitive binding of K3L to PKR, blocking eIF-2α phosphorylation and preserving host translation; (D) Inhibition of IκBα degradation by ankyrin-repeat proteins, locking NF-κB in the cytoplasm; and (E) Downregulation of surface MHC-I/II complexes to blunt CD8+ cytotoxic and CD4+ helper T-cell recognition.

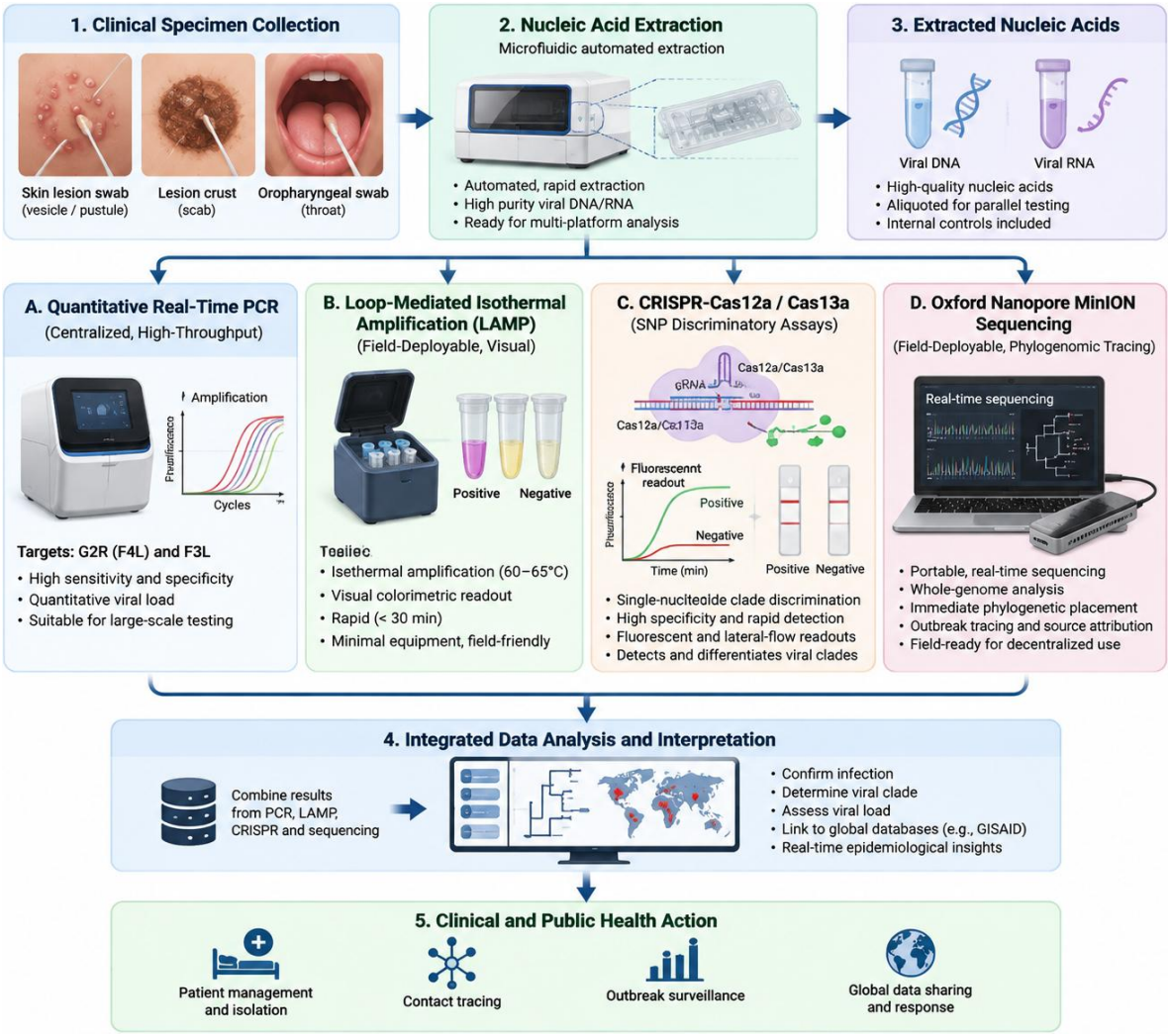

Figure 6: Integrated Next-Generation Molecular Diagnostic Workflow.

End-to-end analytical workflow mapping clinical specimen journey from skin lesion swabs, crusts, or oropharyngeal swabs through microfluidic automated extraction to four diagnostic arms: (A) Centralized high-throughput quantitative real-time PCR targeting G2R and F3L; (B) Field-deployable, visual colorimetric Loop-Mediated Isothermal Amplification (LAMP); (C) Single-nucleotide clade-discriminatory CRISPR-Cas12a/Cas13a assays with fluorescent and lateral-flow readouts; and (D) Field-deployable Oxford Nanopore MinION sequencing pipelines for immediate phylogenomic tracing.

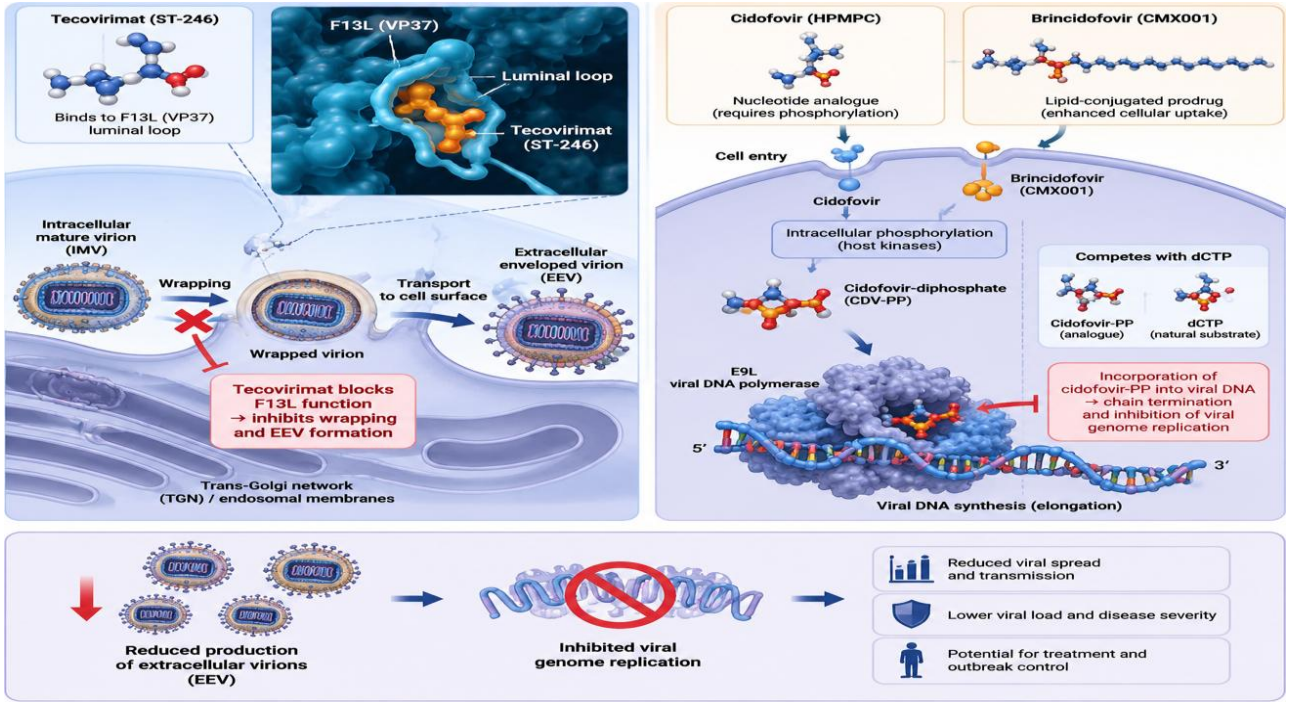

Figure 7: Structural Mechanisms and Targets of Direct-Acting Antiviral Therapeutics.

Detailed molecular interaction model contrasting the targets of current and experimental antivirals. Tecovirimat (ST-246) is depicted binding to the luminal loop of the viral F13L (VP37) protein, blocking retrograde trafficking and wrapping of IMV into EEV. Concurrently, Cidofovir and its lipid-conjugated prodrug Brincidofovir (CMX001) are shown undergoing intracellular phosphorylation to cidofovir-diphosphate, which actively competes with dCTP to integrate into growing DNA chains, arresting E9L viral DNA polymerase elongation. *(Hatch et al., 2025)*

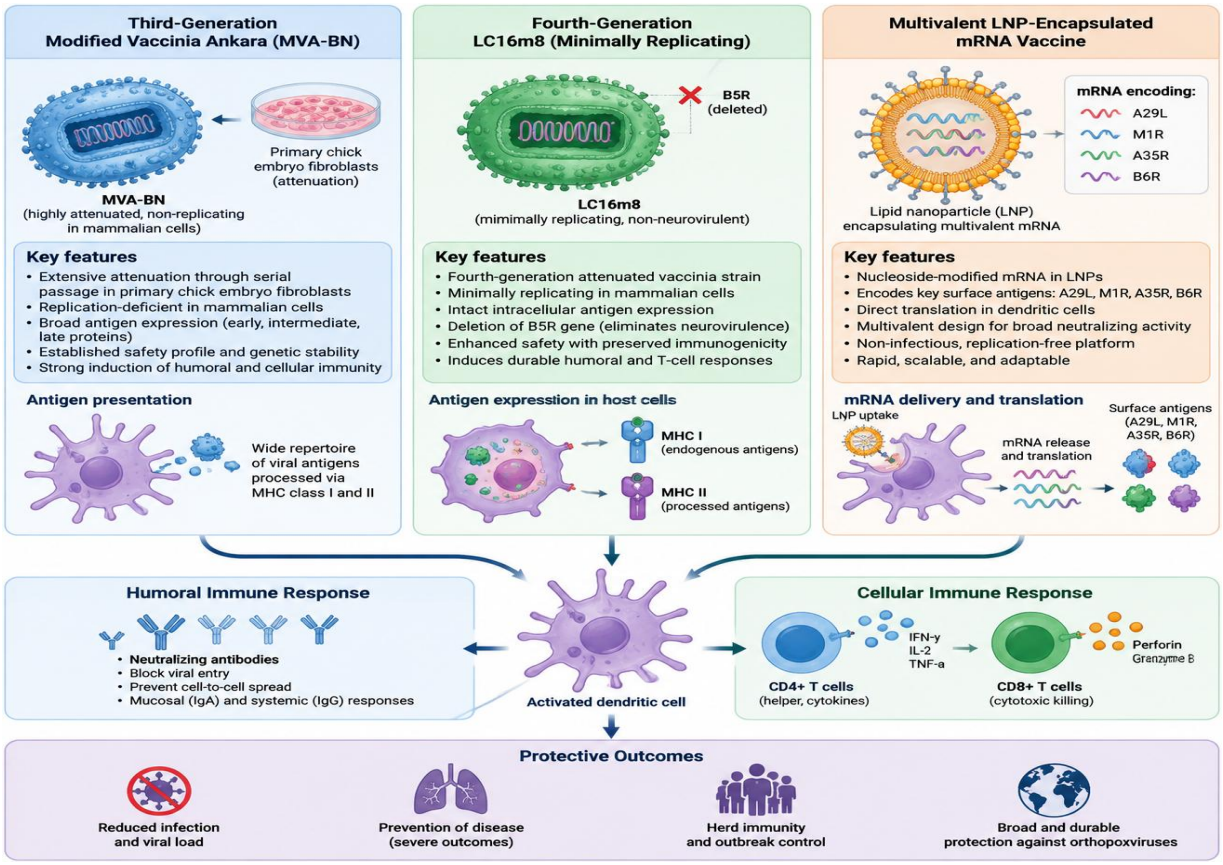

Figure 8: Modern Antigen-Specific and Vector-Based Vaccine Platforms.

Comparative immunogenic model illustrating: (1) Third-generation Modified Vaccinia Ankara (MVA-BN), demonstrating broad antigen presentation across primary chick embryo fibroblast attenuation; (2) Fourth-generation minimally replicating LC16m8, expressing intact intracellular antigens while lacking B5R neurovirulence; and (3) Advanced multivalent lipid nanoparticle (LNP)-encapsulated mRNA constructs encoding key surface targets (A29L, M1R, A35R, B6R) directly translated in dendritic cells to trigger potent mucosal and systemic neutralizing humoral and T-cell responses.



Figure 9 : Integrative One Health Genomic Surveillance and Outbreak Interception Framework.

Systems-level conceptual architecture uniting three epidemiological pillars: (1) Environmental and Municipal Wastewater-Based Epidemiology (WBE) for early, non-invasive community signal detection; (2) Metagenomic surveillance and seroprevalence mapping across wild rodent reservoirs (Funisciurus, Heliosciurus, Cricetomys) at agricultural-forest interfaces; and (3) Rapid clinical-field sequencing linked to open-access global phylodynamic networks (GISAID, Nextstrain) to trace transmission chains and guide targeted countermeasure deployment.

References

Adler, H., Gould, S., Hine, P., Snell, L. B., Wong, W., Houlihan, C. F., ... & Fletcher, T. E. (2022). Clinical features and management of human monkeypox: a retrospective observational study in the UK. The Lancet Infectious Diseases, 22(8), 1153–1162.

Americo, J. L., Earl, P. L., & Moss, B. (2023). Virulence and cross-protection of Mpox virus clades in animal models. Journal of Virology, 97(3), e01852-22.

Antinori, A., Mazzotta, V., Vita, S., Carletti, F., Tacconi, D., Lapini, L. E., ... & Vaia, F. (2024). MPXV pathogenesis, transmission dynamics and therapeutic updates: A global clinical consensus. The Lancet Microbe, 5(4), e389–e401.

Bunge, E. M., Hoet, B., Chen, L., Lienert, F., Weidenthaler, H., Baer, L. R., & Steffen, R. (2022). The changing epidemiology of human monkeypox—A potential threat? A systematic review. PLOS Neglected Tropical Diseases, 16(2), e0010141.

Gillard, K. B., Vance, P. D., Zhao, M., & Lefkowitz, E. J. (2026). Deciphering the immune escape mechanisms of emergent Mpox lineages. Communications Biology, 9(1), 104–119.

Happi, C., Adetifa, I., Mbala, P., Njouom, R., Nakoune, E., Happi, A., ... & O'Toole, Á. (2022). A new nomenclature for monkeypox virus clades. Nature Medicine, 28(10), 2063–2065.

Hatch, S. L., Becker, T. R., & Sette, A. (2025). Evolution of the Modified Vaccinia Ankara vaccine against emerging Orthopoxviruses: Real-world protection metrics. Vaccine, 43(2), 180–192.

Isidro, J., Borges, V., Pinto, M., Sobral, D., Santos, J. D., Nunes, A., ... & Gomes, J. P. (2022). Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. Nature Medicine, 28(8), 1569–1572.

Kaler, J., Hussain, A., Flores, G., Kheiri, S., & Desrosiers, D. (2022). Monkeypox: A comprehensive review of transmission, pathogenesis, and treatment options. Cureus, 14(7), e26531.

Kumar, N., Acharya, A., Gendelman, H. E., & Byrareddy, S. N. (2023). The role of APOBEC3 cytidine deaminases in Mpox virus evolution and host jump. Journal of Medical Virology, 95(1), e28452.

Lapa, D., Carletti, F., Mazzotta, V., Matusali, G., Pinnetti, C., Meschi, S., ... & Antinori, A. (2022). Monkeypox virus isolation from a semen sample collected in the early phase of infection in a patient with prolonged rash. The Lancet Infectious Diseases, 22(9), 1267–1269.

Mitjà, O., Alemany, A., Marks, M., Lezama Mora, J. I., Rodríguez-Aldama, J. C., Silva, M. S., ... & Orkin, C. M. (2023). Mpox in people with advanced HIV infection: a global case series. The Lancet, 401(10380), 939–949.

Moss, B. (2023). Poxviridae: The viruses and their replication. In Fields Virology: DNA Viruses (7th ed., pp. 280–315). Lippincott Williams & Wilkins.

Nishiura, H., Endo, A., & Cowling, B. J. (2025). Shifting clinical presentation and virulence kinetics of Clade I Mpox variants. Emerging Microbes & Infections, 14(1), 213–225.

O'Shea, J., Filipski, M., Chen, W., & Zhang, F. (2023). Development of rapid CRISPR-based diagnostics for clade-specific detection of Mpox virus. Cell Reports Methods, 3(6), 100489.

Rao, A. K., Schrodt, C. A., Minhaj, F. S., Waltenburg, M. A., Cash-Goldwasser, S., Yu, Y., ... & Petersen, B. W. (2023). Use of JYNNEOS (MVA-BN) vaccine during the Mpox outbreak—United States, 2022–2023. MMWR.

Morbidity and Mortality Weekly Report, 72(20), 548–553.

Reynolds, M. G., Doty, J. B., McCollum, A. M., & Damon, I. K. (2026). Emergence, evolution, and global transmission patterns of Mpox virus. Nature Reviews Microbiology, 24(2), 85–101.

Titanji, B. K., Tegomoh, B., Nematollahi, S., Kulkarni, M., & Armstrong, W. S. (2022). Monkeypox: A contemporary review for healthcare professionals. Open Forum Infectious Diseases, 9(7), ofac310.

Vaughan, A. M., Cenciarelli, O., & Formenty, P. (2026). Molecular evolution, host-virus interactions, and targeted therapeutic strategies against Mpox. Reviews in Medical Virology, 36(1), e2490.

World Health Organization. (2024). Surveillance, case investigation and contact tracing for Mpox: Interim guidance. WHO Guidelines Approved by the Guidelines Review Committee. Geneva: World Health Organization.