Exemplified mRNA MPox vaccine pipeline

Created for the Alliance for Cruelty Free Science

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While no fully 100% animal-free mpox vaccine has been approved or completed clinical trials as of August 29, 2025, advancements in mRNA technology, artificial intelligence (AI), and non-animal testing methods—supported by regulatory changes like the FDA Modernization Act 2.0—make such a pipeline feasible.

This act allows alternatives to animal testing for drug and vaccine approval when scientifically valid, including in silico modeling, organ-on-a-chip systems, and human-based assays, potentially phasing out traditional animal requirements for certain biologics like vaccines.

Emerging mRNA-based mpox vaccine candidates, such as multivalent or penta-component designs, can leverage these tools for rapid, ethical development without animal-derived materials or testing.

Below is an exemplified pipeline for an mRNA-based mpox vaccine, drawing from current research and non-animal alternatives used in fields like COVID-19 vaccine development. This approach avoids animal models entirely by relying on computational, cellular, and human-derived systems.

1. Antigen Identification and Selection (In Silico Phase)

- Use bioinformatics and AI algorithms to analyze the mpox virus (MPXV) genome, predicting immunogenic antigens like A35R, M1R, B6R, A29L, or H3L based on sequence data from databases (e.g., NCBI or GISAID).
- Tools like epitope prediction software (e.g., IEDB or Vaxign) and machine learning models simulate human immune recognition without physical experiments.

Output: A multivalent design encoding 4–5 antigens for broad protection against clades I and II.

Timeline: 1–2 weeks; fully computational, no animals or cells needed yet.

2. Vaccine Design and Optimization (In Silico and In Vitro Synthesis)

- Design mRNA sequences using codon optimization software to encode selected antigens, incorporating lipid nanoparticle (LNP) formulations for delivery—similar to those in COVID-19 mRNA vaccines.
- Simulate stability, translation efficiency, and immune activation via molecular dynamics software or AI platforms like AlphaFold for protein structure prediction.

Timeline: 2–4 weeks; ethical and scalable.

3. In Vitro Efficacy and Immunogenicity Testing

- Transfect human cell lines (e.g., HEK293 or immune-derived cells like dendritic cells from human blood donations) to confirm antigen expression via assays like Western blot or flow cytometry.
- Assess immune response using human peripheral blood mononuclear cells (PBMCs) or engineered immune cells to measure cytokine production, T-cell activation, and neutralizing antibody generation—mimicking human immunity without animals.

- Use high-throughput screening to optimize dosage and formulation.

Output: Data showing robust humoral (antibody) and cellular immunity, comparable to cross-protective responses seen in mRNA mpox candidates.

Timeline: 4–8 weeks; fully human-cell-based.

4. Advanced Preclinical Safety and Efficacy Evaluation (Non-Animal Models)

- Employ organ-on-a-chip or 3D bioprinted human tissue models (e.g., skin, lung, or immune organoids) to simulate MPXV infection and vaccine protection, assessing viral entry inhibition and tissue-level responses.
- Use "virtual human" simulations or AI-driven pharmacokinetic/pharmacodynamic (PK/PD) modeling to predict toxicity, biodistribution, and long-term effects.
- Incorporate microphysiological systems for multi-organ interactions, as used in COVID-19 research, to evaluate off-target effects without animals.

Output: Safety profile confirming low reactogenicity and predicted 80–90% efficacy based on surrogate markers like neutralizing antibodies.

Timeline: 8–12 weeks; aligns with FDA's roadmap for reducing animal use.

5. Regulatory Submission and Clinical Trials

- Submit data to regulators (e.g., FDA, EMA, or WHO) under frameworks allowing non-animal alternatives, emphasizing human-relevant evidence

- Phase 1: Small human trials (10–100 volunteers) for safety and immunogenicity.
- Phase 2: Expanded trials (hundreds) to refine dosing and confirm immune responses.
- Phase 3: Large-scale efficacy trials (thousands) in at-risk populations, using correlates of protection (e.g., antibody levels) or human challenge models if ethically approved.

Output: Potential approval as a two-dose regimen, similar to JYNNEOS but with enhanced mRNA-driven protection.

Timeline: 6–18 months, accelerated via emergency use pathways.

This pipeline could reduce development time to under a year and costs significantly, while being cruelty-free and more predictive of human outcomes.

Challenges include validating non-animal models for full regulatory acceptance, but ongoing mpox mRNA research demonstrates feasibility.

For real-world implementation, collaborate with organizations who have mRNA expertise.

References for the mRNA Mpox Vaccine Pipeline

Below is a compiled reference list of sources that support the key elements of the exemplified pipeline, including regulatory changes for non-animal testing, mRNA mpox vaccine development, specific antigens, computational tools, and alternative testing methods. These are drawn from recent research and publications as of early 2026.

Regulatory Frameworks and Alternatives to Animal Testing

The FDA Modernization Act 2.0 enables alternatives to animal testing for drug and vaccine approval, emphasizing scientifically valid non-animal methods.

FDA. (2025). FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs.
https://www.fda.gov/news-events/press-announcements/fda-announces-plan-phase-out-animal-testing-requirement-monoclonal-antibodies-and-other-drugs

The Act allows for the use of non-animal methods in regulatory submissions, potentially phasing out traditional animal requirements for biologics like vaccines.

Arrowsmith, J., et al. (2023). The FDA Modernisation Act 2.0: Bringing Non-Animal Technologies to the Regulatory Table. Drug Discovery Today.
https://www.sciencedirect.com/science/article/abs/pii/S1359644623000120

FDA's roadmap outlines a 3–5 year plan to reduce animal testing in preclinical safety studies using alternatives like in silico modeling and organ-on-a-chip systems.

BioIVT. (2025). FDA Roadmap to Reducing Animal Testing: A New Regulatory Era.

https://bioivt.com/blogs/fda-roadmap-to-reducing-animal-testing-a-new-regulatory-era

mRNA Mpox Vaccine Candidates

Multivalent mRNA vaccines encoding multiple mpox antigens show strong immunogenicity and protection in preclinical models.

Liu, R., et al. (2025). An Mpox Multi-Antigen-Tandem Bivalent mRNA Candidate Vaccine Effectively Protects Mice Against the Vaccinia Virus. PMC.
https://pmc.ncbi.nlm.nih.gov/articles/PMC12031407/

Next-generation mRNA mpox vaccine programs, including multivalent designs, are advancing through clinical trials for broader protection.

CEPI. (2025). Stronger, Faster, Fairer: Making Mpox Vaccines Accessible.
https://cepi.net/stronger-faster-fairer-making-mpox-vaccines-accessible

Research on multivalent mRNA vaccines like AAL (encoding three antigens) and AALI demonstrates robust immune responses and protection against mpox.

Liu, R., et al. (2025). A Multivalent mRNA Vaccine Elicits Robust Immune Responses and Confers Protection in a Murine Model of Monkeypox Virus Infection. Nature Communications.
https://www.nature.com/articles/s41467-025-61699-w

Mpox Virus Antigens (A35R, M1R, B6R, A29L, H3L)

Antigens A29L, M1R, A35R, and B6R are immunogenic and provide protection against mpox when used in recombinant vaccines.

Li, J., et al. (2024). Exploring Monkeypox Virus Proteins and Rapid Detection Techniques. PMC. https://pmc.ncbi.nlm.nih.gov/articles/PMC11165096/

Trivalent vaccines using A35R, M1R, and B6R elicit efficient neutralizing antibodies and protect against mpox challenge.

Zhang, N., et al. (2024). Single-Chain A35R-M1R-B6R Trivalent mRNA Vaccines Protect Mice from Monkeypox Virus Challenge. eBioMedicine. https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(24)00428-6/fulltext

Subunit vaccines composed of A29L, M1R, A35R, and B6R induce strong humoral and cellular immunity against mpox.

Zeng, J., et al. (2023). A Subunit Vaccine Candidate Composed of Mpox Virus A29L, M1R, A35R, and B6R Modified Proteins Elicits Robust Immune Responses in Mice. Vaccines. https://pmc.ncbi.nlm.nih.gov/articles/PMC10537547/

Antigens like A35R, A29L, B6R, H3L, and M1R play key roles in viral replication and are targets for vaccine development.

Sino Biological. (2025). Mpox Virology and Drug Development: A Focus on Key Viral Targets. https://www.sinobiological.com/category/ads/mpox-proteins

Non-Animal Testing Methods (In Vitro, Organ-on-a-Chip)

Organ-on-a-chip technologies mimic human organs for drug and vaccine testing, reducing reliance on animal models.

Wyss Institute. (2025). Human Organs-on-Chips. https://wyss.harvard.edu/technology/human-organs-on-chips/

https://www.sciencedirect.com/science/article/pii/S2468202021000188

AI, organoids, and organ-on-a-chip methods improve preclinical vaccine testing, bridging the gap to human trials without animals.

Kaushik, G., et al. (2025). Artificial Intelligence-, Organoid-, and Organ-on-Chip-Powered Models to Improve Pre-Clinical Animal Testing of Vaccines and Immunotherapeutics. Frontiers in Artificial Intelligence. https://www.frontiersin.org/journals/artificial-intelligence/articles/10.3389/frai.2025.1681106/full

Computational Tools (AlphaFold, IEDB, Vaxign)

AlphaFold predicts protein structures for vaccine design, aiding in antigen optimization and stability simulation.

Jumper, J., et al. (2023). AlphaFold2 and Its Applications in the Fields of Biology and Medicine. Signal Transduction and Targeted Therapy. https://www.nature.com/articles/s41392-023-01381-z

IEDB provides tools for epitope prediction and analysis, supporting antigen selection in vaccine development.

IEDB. (2025). IEDB.org: Free Epitope Database and Prediction Resource. https://www.iedb.org/

Vaxign uses reverse vaccinology for vaccine target prediction based on genomic data.

VIOLIN. (2025). Vaxign Tutorial - Vaccine Design. https://violinet.org/vaxign/tutorial/index.php