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Immunogenic proteins and potential delivery platforms for mpox virus vaccine development: A rapid review

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Abstract

Since May 2022, the mpox virus (MPXV) has spread worldwide and become a potential threat to global public health. Vaccines are important tools for preventing MPXV transmission and infection in the population. However, there are still no available potent and applicable vaccines specifically for MPXV. Herein, we highlight several potential vaccine targets for MPVX and emphasize potent immunogens, such as M1R, E8L, H3L, A29L, A35R, and B6R proteins. These proteins can be integrated into diverse vaccine platforms to elicit powerful B-cell and T-cell responses, thereby providing protective immunity against MPXV infection. Overall, research on the MPXV vaccine targets would provide valuable information for developing timely effective MPXV-specific vaccines.

Keywords: MPXV, Vaccine, Targets, Epitopes, Platforms

Introduction

As of March 10th 2023, the Center for Disease Control and Prevention (CDC) reported 86231 mpox virus (MPXV) cases worldwide. A total number of 84,470 cases were reported by CDC in 103 locations that have not historically reported mpox infection, such as the United States, Brazil, and Spain. Sexual transmission is the main route of the current mass spread of MPXV (1). Monkeypox (mpox) is a self-limiting disease with clinical symptoms including fever, headache, lymphadenopathy, and myalgia (1). Viral culture, electron microscopy, immunohistochemistry, serum IgG and IgM assays, and PCR techniques are several methods for the diagnosis of MPXV (2). The CDC recommends that people considered at high risk of MPXV infection should receive a vaccination against the virus. However, while existing smallpox vaccines (ACAM2000, JYNNEOS, and LC16m8) provide protection against MPXV infection, the risk of breakthrough infection and side effects suggest that safer MPXV-specific vaccines should be developed (3). To better design and develop MPXV vaccines, an understanding of MPXV structure, genome, and life cycle is necessary. We emphasize the potential vaccine targets, such as M1R, E8L, H3L, A29L, A35R, and B6R proteins, and highlight applicable platforms, such as multiepitope subunit-, mRNA and DNA-, viral vector-, and nanoparticles-based vaccines, which are vital and valuable for designing and developing MPXV-specific vaccines.

The genome and structure of MPXV

MPXV belongs to the orthopoxvirus genus, poxviridae family, which also includes variola virus (VARV), cowpox virus (CPXV), vaccinia virus (VACV), etc. VACV is the experimental prototype of the poxvirus family. The length of the MPXV DNA genome is about 197,205 bp, encoding approximately 190 proteins (4). Its genome consists of a large conserved central genomic region and two variable regions on both ends (5) (Figure. 1A). The nucleotide sequence of the viral core region is 96.3% identical with that of VACV, which encodes essential enzymes such as DNA polymerases and capping enzyme, and structural proteins such as M1R, E8L, H3L, A29L, A33R, B6R proteins (6). The left and right variable arms encode proteins that associate with viral virulence and host-range factors (4). These regions also include two same but oppositely oriented sequences (6438 bp), called inverted terminal repeats (ITR), which are the hot spots for mutation during evolution (7). For instance, the ITR can be preferentially mutated by the host apolipoprotein B mRNA-editing, catalytic polypeptide-like 3 (APOBEC) enzyme, which is essential for MPXV evolution and potential human adaptation (7, 8).

As information about MPXV is scarce, when analyzing the MPXV, we draw parallels from VACV or other orthopoxviruses. The average size of MPXV is approximately 360 nm × 270 nm × 250 nm, with a brick-shaped or oval morphology (9) (Figure. 1B). Intracellular mature virions (IMV) and extracellular enveloped virions (EEV) are two major forms of infectious virions during MPXV replication cycle. The surface tubular proteins of virion are usually in the shape of regular 10 nm long protrusions (10). Membrane and envelope

proteins located in the outer layer of virion are essential for virion attachment to host cells. Hence, these proteins can potentially serve as attractive targets for developing MPXV-specific vaccine candidates. A typical dumbbell-shaped nucleoprotein core, separated by a semipermeable shell, contains a large double-stranded linear DNA genome and enzymes, such as the RNA polymerase, and polyA polymerase necessary for viral early gene expression (11, 12). Protein-rich lateral bodies in virions can work immediately once the virus enters the host cell, offering an optimal intracellular environment for virus replication (13). For instance, the viral H1 phosphatase from the lateral bodies can inhibit interferon-gamma (IFN-γ) signaling by dephosphorylating signal transducer and activator of transcription 1 (STAT1) (14).

The life cycle of MPXV

The process of MPXV infection consists of four stages: viral particle entry, fusion, replication, and release (Figure 2). Orthopoxvirus entry and fusion rely on the entry fusion complex (EFC), which is highly conserved in all poxviruses (15). It consists of 4 proteins required for attachment, such as A27L, H3L, D8L, and A26L, and the other 11 proteins required for membrane fusion and core entry, such as A16, A21, and A28 (16-19). A27L, H3L, and D8L proteins of VACV binding to glycosaminoglycans (GAGs) and A26L binding to extracellular matrix protein laminin initiate VACV attachment to enable viral entry (10, 20-23). The GAGs, commonly expressed on the cell surface, are a family of sulfated polysaccharides. The GAGs family also includes heparan sulfate (HS), chondroitin

sulfate (CS), etc. (24). The A29L protein of MPXV, as a homologous protein of A27L in VACV, contributes to the viral attachment to the host cell membrane. A29L protein exhibits a strong affinity with HS and HS-derived oligosaccharides by surface plasmon resonance (SPR) (10). Then, IMV and EEV of orthopoxvirus activate the host cell macropinocytosis for internalization (25). However, these two forms of viruses activate macropinocytosis and fusion by different mechanisms, EEV exposes the EFC by shedding the outer envelope, while IMV inactivates fusion inhibitory entry fusion complex proteins that are not present in EEV (25). Early proteins encoded by egressed nascent mRNA in semi-permeable viral core wall facilitate viral core uncoating (26). In addition, these proteins can act as transcription factors required for intermediate gene transcription and enzymes required for viral genome replication such as DNA polymerases (9). Robust intermediate and late DNA replication occurs in large replication factories wrapped by the endoplasmic reticulum membrane (27). Interestingly, the DNA polymerase holoenzyme of MPXV ensures high DNA replication efficiency by enclosing single-stranded template DNA (28). The virus undergoes assembly and morphogenesis in the cytoplasmic structure (29). IMV is encapsulated by a double membrane from the Golgi apparatus, forming intracellular enveloped virus (IEV) (30). IEV uses microtubules to move from the site of membrane wrapping in Golgi to the cell surface and fuses with the plasma membrane to form cell-associated enveloped viruses (CEV) (31, 32). Then, CEV triggers actin tail formation that propels virions out of cells, which are called EEV (31). A deep understanding of MPXV biology can help accelerate the development of new generation vaccines.

Effectiveness of currently available smallpox vaccines against MPXV

Considering that MPXV and VACV are genetically similar (96.3%), cross-reactive antibodies produced by the smallpox vaccine can provide indirect protection against MPXV infection (6). Therefore, vaccines that were developed against smallpox are now being used as approaches to prevent MPXV spread in certain countries. There are three vaccines, ACAM2000, JYNNEOS, and LC16m8, currently available for MPXV (Table 1). Here, we discuss the characteristics of these smallpox vaccines and their effectiveness against MPXV in animals and humans.

ACAM2000

ACAM2000 (second generation) is a live plaque-purified VACV derivative of Dryvax (first generation) and was licensed by Food and Drug Administration (FDA) in August 2007 for immunization against smallpox disease in individuals (33). ACAM2000 vaccine, containing replication-competent VACV, can cause serious side effects (like myopericarditis and pericarditis) in immunocompromised patients (34). ACAM2000 vaccination in immunosuppressed individuals (e.g., HIV-infected persons) was explicitly prohibited according to CDC guidelines. For instance, in 2022, HIV-positive cases account for 54.29% of all 124 MPXV confirmed cases in Italy, Australia, the Czech Republic, etc. (35). Therefore, the ACAM2000 vaccine seems not to be a perfect and ideal tool for the prevention of current MPXV transmission due to its undesired side effects.

JYNNEOS

JYNNEOS (IMVAMUNE, IMVANEX, MVA-BN) is a third-generation vaccine based on the non-replicating modified VACV Ankara (MVA) strain and licensed by FDA for the prevention of smallpox and mpox in adults in 2019 (36). JYNNEOS is the preferred choice due to fewer side effects and contraindications compared with ACAM2000. In response to the unprecedented mpox outbreak in 2022, the FDA issued an emergency use authorization of JYNNEOS on August 09, 2022. However, a two-shot JYNNEOS vaccination produces relatively low levels of MPXV-neutralizing antibodies in healthy individuals, with poor neutralizing capacity (37). Furthermore, breakthrough infection with MPXV was reported in 90 patients who received a single dose of JYNNEOS on June 28, 2022 (38). In a retrospective observational study in Lyon, France, from June to August 2022, 11 (10%) cases of breakthrough MPXV were observed among 108 adults who received one dose of JYNNEOS after exposure to MPXV (39). 5 patients with severe mpox symptoms after post-exposure (1 patient) preventive vaccination or pre-exposure (4 patients) preventive vaccination with the JYNNEOS vaccine were reported (40). What's more, 8 cases were tested as MPXV positive 14 days post vaccination while 32 cases tested positive 0-13 days post vaccination (41). In summary, the protection of JYNNEOS against MPXV infection in humans is relatively low.

LC16m8

LC16m8 is another third-generation vaccine containing a virus derived from the Lister strain of vaccinia in rabbit kidney cells (42). In 1980, LC16m8 is licensed for use against biological terrorism by Japanese Regulatory Authorities (43). The virus in LC16m8 is attenuated as it was developed to lack the B5R envelope protein gene, and its ability to replicate in vaccine recipients is restrictive (42). LC16m8 generates neutralizing antibody titers (>1:40) to multiple orthopoxviruses and broad T-cell responses in vitro (44). Similarly, vaccination with LC16m8 can provide longer than one year of protection against MPXV in monkeys (45). However, the effectiveness of LC16m8 against MPXV in humans has not been reported so far.

Development of MPXV-specific vaccines and potential vaccine targets

In this part, we highlight the proteins on the membrane and envelope as potential targets (Table 2) (Figure 3), as well as propose feasible platforms to deliver these immunogens to develop new generation of MPXV vaccines.

Membrane protein targets on IMV

M1R

The MPXV M1R is the homologous protein of VACV L1R. VACV L1R is located on the membrane of IMV. L1R outer domain, facing the cytoplasm in intracellular viruses, contains three intramolecular disulfide bonds (46). The L1R attaches to the cell surface by

binding to non-GAG molecules on the cell surface and adheres to the viral membrane via a C-terminal transmembrane anchor (47, 48). A myristylated domain in the N-terminal region of L1R is the target of potent neutralizing monoclonal antibodies (7D11), which prevents VACV entry into BSC-1 cells in a dose-dependent fashion (49). L1R protein is the target of neutralizing monoclonal antibody (mAb), named 2D5 (49). Monoclonal neutralizing antibody (7D11, 10F5) binds L1R in a conformation-specific manner, these antibodies identify discontinuous epitope carrying two loops that are anchored together by a disulfide bond (50). Hence, the proper folding of the L1R protein is vital for the induction of neutralizing antibodies, which should be considered in the next generation of L1R based vaccines (50). In theory, homologous M1R can be considered to be integrated into the MPXV vaccine development.

E8L

VACV D8L protein (304aa) contains 3 regions: virion surface (1–275 aa), transmembrane (276–294 aa) and short intra-virion (295–304 aa) regions. D8L protein exposed on the viral surface is composed of human carbonic anhydrases homology (CAH) in the large N-terminal and an uncharacterized C-terminal domain. The CAH can bind the GAGs on the host cell surface, which is important for VACV entry (51). Neutralizing mAb (named LA5) binds D8L protein and can neutralize VACV to block infection in the presence of complement (51). D8L activates dendritic cells (DCs) and induces the secretion of cytokines IL-12p70, IL-10, TNF-α, and IL-6 (52). The viral membrane protein E8L in

MPXV, a homologous protein of D8L, is essential for virion attachment to host cells. Poxviruses routinely rely on host cell surface lipid rafts enriched with negatively charged gangliosides to enter host cells (53). Importantly, MPXV E8L possesses a ganglioside-binding domain that overlaps with three potential linear B-cell epitopes (54). Therefore, employing these epitopes from the E8L protein in the MPXV vaccine, in theory, produce potent neutralizing antibodies. These characteristics of the E8L protein potentially enable it as an attractive immunogen when considering vaccine targets.

H3L

The VACV H3L protein can be attached to the IMV membrane by its C-terminal hydrophobic structure and bind to HS on the cell surface, promoting viral entry (21, 55). H3L knock out viruses exhibit 10-fold lower viral titers and show less infectious than wild-type virions (21). More importantly, the H3L protein is essential for triggering host cells to produce T- and B- cell immune responses in host cells. For instance, the H3L protein is a target of cellular immune responses, and the protein exhibits at least two identified human leucocyte antigen (HLA) class I-restricted T-cell epitopes which can elicit robust IFN-γ response (56). H3L protein activates and matures DCs to secrete the cytokines IL-12p70, IL-10, TNF-α, and IL-6, then stimulated DCs induce CD8+ T cells to proliferate and secrete IFN-γ, thereby killing the virus-infected cells (52). Most neutralization epitopes across between 1 and 239aa of H3L protein (57). In addition, recombinant H3L protein-immunized mice produce a high titer of neutralizing antibodies (IC50 = 760)

against VACV, thus resisting intranasal challenges with lethal doses of the VACV (58). Hence, the H3L protein is one of the key targets for MPXV vaccine design.

A29L

MPXV A29L protein is a homolog of VACV A27L. The C-terminal leucine zipper structural domain (80 to 101 aa) of A27L protein attaches to the viral membrane, whereas the N-terminal region (21 to 32 aa) enables attachment to host cells via its GAGs-binding domain (59). The A27L protein is required for both IMV transport and the process of envelopment that leads to IEV formation (60). Peptides of 24-39 aa, 45-59 aa, and 77-91 aa from A27L protein were identified as CD4+ T cell epitopes, while peptide of 89-103 aa was identified as CD8+ T cell epitopes (61). The epitope (21 to 49 aa) on the MPXV A29L protein, adjacent to the GAGs binding region, is a target of a monoclonal antibody (named 69-126-3-7) (62). In summary, vaccines targeting the MPXV A29L protein can be developed to interfere with viral adhesion to the host cell surface mediated by neutralizing viral particles.

Envelope protein targets on EEV

A35R

A35R is VACV A33R ortholog in MPXV. VACV A33R protein is expressed on the surface of EEV but not IMV (63). A33R protein is a type II integral membrane glycoprotein with a

hypothetical C-type lectin domain (64). Moreover, a three-protein complex composed of A33R, A34R, and B5R is critical for the efficient production of infectious EEV, which is also involved in endoplasmic reticulum exit and proper localization to the intracellular site of wrapping (65). In addition, the A33R protein also regulates the interaction of A36R with microtubule motor proteins (66). A33R and B5R peptides can elicit a robust T cell response in multiple healthy donors by using binding assays to multiple HLA-DR molecules and T cell stimulation assays (67). In addition, A33R is a target for neutralizing antibody responses against EEV in the presence of complement (68). Despite high homology between A33R in VACV and A35R in MPXV, those differences between proteins can impact the cross-protection of smallpox vaccines against MPXV. For instance, mAb (named 1G10) can bind to VACV A33R with high affinity, however, it cannot bind to the MPXV A35R (69). Hence, developing a vaccine against antigens from MPXV instead of using the traditional smallpox vaccine will bring stronger protection against mpox.

B6R

B6R protein in MPXV is the homologous protein of VACV B5R. B5R is a type I glycoprotein with a large extracellular domain consisting primarily of four short consensus repeats (70). B5R protein is essential for wrapping IMV and for EEV to induce the formation of actin tails on the host cell surface expressing A33 and A36 proteins (71, 72). The EEV B5R is the major target of EEV-neutralizing antibodies after smallpox vaccination as demonstrated by antibody depletion experiments (73). Treatment of a

combination of anti-B5R and anti-L1R mAbs significantly prolonged survival rate and reduced viral load at the scar site after challenging VACV in mice (74). Mechanically, the protection provided by these antibodies is not primarily from direct neutralization effects, but rather heavily dependent on the ability of anti-B5R mAb to recruit complement (C3 and C1q) (75-78).

Potential vaccines integrating multi-proteins

Notably, vaccines targeting both IMV and EEV immunogens are more protective than anti-IMV or EEV antigens alone (79, 80). In addition, CD4+ T cells allow heterotypic help for B cells to elicit potent humoral responses against multiple VACV proteins (A27L, A33R, B5R, and L1R) in VACV immunized donors (81). Vaccination of BALB/c mice with Escherichia coli-expressed VACV proteins A27L, B5R, and D8L protects mice from lethal VACV challenge (82). In the intravenous nonhuman primate MPXV model, the subunit vaccine (named 4pox) targeting A33R, B5R, A27L, and L1R, can protect mice from lethal MPXV infection after two doses of vaccination (83). Moreover, a DNA vaccine targeting the VACV L1R, A27L, B5R, and A33R proteins can protect nonhuman primates against MPXV-induced severe disease (84-86). Hence, multiple proteins of MPXV can be integrated for vaccine targets.

Different platforms based on the MPXV antigens

Multiple platforms are available for designing specific candidates against MPXV. These

include the multiepitope subunit-, DNA and mRNA-, viral vector-, and nanoparticles-based vaccines (87) (Figure 4).

Multiepitope subunit vaccine

Subunit vaccines make up the highest percentage of vaccines developed (88). Multiepitope subunit vaccines, being easier to manufacture, is largely depending on immunogen without additional vector elements. Therefore, it is more suitable for immunocompromised individuals

(eg. HIV patients) than viral vector-based vaccines. Adjuvants are important and necessary in the development of subunit vaccines, peptides that covalently coupled with adjuvants such as aluminum phosphate and cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) can activate strong immune responses (89, 90). Interestingly, novel multiepitope-based subunit vaccine candidates against the MPXV were developed in several studies (91-94). However, preclinical toxicology studies and clinical trials of these vaccine candidates require further refinement. Importantly, the prerequisites of developing such vaccines are to employ bioinformatics to predict cytotoxic T-lymphocyte, helper T-lymphocyte, and B-cell epitopes on the target proteins mentioned above. Interestingly, a new multi-epitope vaccine, named S7M8, can prevent SARS-CoV-2 and MPXV co-infection (95). The development of a highly effective multiepitope-based subunit vaccine to prevent MPXV infection is possible and could serve as a safer and more effective alternative than traditional live vaccines such as ACAM2000 and JYNNEOS (96).

DNA and mRNA-based vaccines

During the COVID-19 pandemic, the DNA and mRNA-based vaccines, particularly the mRNA vaccine, played an important role against SARS-CoV-2 (97). The DNA and mRNA vaccines carry and express the target proteins after being injected into hosts, thereby eliciting a cellular and humoral immune response to fight against viral infection. Compared to mRNA vaccines, DNA vaccines are easier to transport and store due to their thermal stability. In addition, the schedule of developing and producing DNA vaccine is shorter and more economical as DNA plasmids can replicate rapidly in E. coli (98). DNA vaccines containing VACV immunogens (L1R, A27L, A33R, B5R), named 4pox, can protect against lethal MPXV challenge in non-human primates (80). Meanwhile, the 4pox vaccination can prevent aerosol-challenged poxvirus infection and alleviate the severity of disease in highly susceptible animals (84). The mRNA does not replicate and interact with the genome in the host. Moreover, the mRNA can be degraded easily and naturally. Therefore, the risk of insertional mutagenesis is low. Two successful mRNA vaccines against the SARS-CoV2, BNT1262b2, and mRNA-1273, have both demonstrated high efficacy in clinical trials (99, 100). However, such mRNA vaccines require cold-chain transport and are relatively costly, which limits distribution in poor and remote areas (101). An urgent issue for mRNA vaccines is to improve their thermal stability to facilitate transportation if this platform is applied to deliver MPXV antigens in the future.

Viral vector vaccine

Compared to conventional subunit vaccines, viral vector vaccines have several advantages, first, they can induce cellular response beyond antibody response, Second, they can induce high immune responses without the use of adjuvants, and in most cases, only single dose usage of such vaccine is required (102). For instance, a single intramuscular injection of an adenoviral vector vaccine delivering the A27L antigen of VACV induced robust and durable cellular and humoral immune responses and protected mice from intranasal challenge with a lethal VACV 4 weeks after vaccination, with protective efficacy lasting for at least 35 weeks (103). Recombinant adenovirus (rAd5) has been shown as the most immunogenic and protective vector with only one dose usage, whereas heterologous prime-booster with sequential recombinant vesicular stomatitis virus (rVSV) and Venezuelan equine encephalitis virus replicons (VRP) was the most immunogenic and protective regimen by comparing the immunogenicity of six different viral vectors inserting the same antigen - CPXV virus 035R (104).

Nanoparticles-based vaccines

Currently, nanoparticles have received much attention in the development of COVID-19 vaccines. The application of nanomaterials has many advantages, for example, it can protect the vaccine active component of vaccine from degradation, improve the solubility of the effective elements, and enhance the transportation of antigens to the right location. In addition, nanoparticle-based vaccines can enhance immune responses due to the

high-density arrangement of antigens and appropriate diameter (105). Nanoparticles with a size smaller than 5 nm can enter the body circulation, while nanoparticles with a size of 5-100 nm can preferentially diffuse to the draining lymph nodes, and nanoparticles larger than 100 nm can reside at the injection site (105). The carriers used for nanoparticle vaccines are classified as organic and inorganic. Organic carrier nanoparticle vaccines mainly include lipid nanoparticles (LNP), and inorganic carriers mainly include virus-like particles (VLP), protein nanoparticles, metal nanoparticles, and carbon nanomaterials (106).

Lipid nanoparticles (LNPs)

Given that LNPs have high biocompatibility and stable property of precise targeting, thus they are often applied to deliver active ingredients, such as DNA, mRNA, and siRNA in vaccine development (107). Specifically, lipid delivery carriers can protect mRNA from engulfment by phagocytes or degradation by nucleases. For instance, MPXVac-097, in which the A29L, E8L, M1R, A35R, and B6R mRNA are wrapped in LNPs for efficient delivery, can elicit MPXV-specific T cell responses and broadly neutralizing antibodies that cross neutralize CPXV, VACV, and MPXV (108).

Liposomes, as important LNPs, are spheres (20-1000 nm) consisting of water nuclei surrounded by phospholipid bilayers (107). Water-soluble substances, such as nucleic acids, can be attached to the water core of liposomes, lipophilic antigens can be incorporated into lipid bilayers, and other types of proteins, such as small-molecule ligands, peptides, or

monoclonal antibodies, can adhere to the surface to form liposomes that can target specific locations in the host. In addition, the toxicity of liposomes is minimal (109). Epaxal, as a liposome-based hepatitis A (HAV) vaccine, was shown to have limited adverse effects and provide efficient protection for up to 20 years in more than 95% of individuals in vaccinated clinical cohorts (110).

Virus-like particles (VLP) and protein nanoparticles

VLP and naturally occurring ferritin can self-assemble to form stable nanoparticles and can deliver protein antigens in high densities on the carrier surface (111). With such advantages, these nanoparticles can facilitate BCR cross-linking and induce more robust humoral responses than naked vaccines, as reported in several previous studies (111-113). Additionally, engineered protein nanoparticles, named I53-50, can carry more multiple antigens than ferritin, enabling mosaic nanoparticle as a useful tool for developing effective broad-spectrum vaccines, which can potentially induce strong immune responses against diverse variants of SARS-CoV-2 (114). In summary, VLP and protein nanoparticles can serve as potential platforms for designing broadly protective vaccines against multiple pathogens, including MPXV.

Metal nanoparticles and carbon nanomaterials

Gold nanoparticles with different sizes can induce respective size-dependent immune signaling pathways. Immunization of gold nanomaterials smaller than 10 nm activated the

NLRP3 inflammasome, caspase-1, leading to IL-1β production, while gold nanoparticles larger than 10 nm tend to activate the NF-kB signaling pathway (115). Therefore, the size of gold nanoparticles should be optimized to activate the most effective immune responses against pathogens (116).

Carbon nanomaterials, mainly including carbon nanotubes (CNT), graphene, fullerenes, and quantum dots, are also potential platforms for MPXV vaccine development (117). Until now, CNT and graphene are well studied and the most common carbon carriers used in vaccine design and development. CNT, as cylinders composed of graphene layers, can allow antigens to be adsorbed onto the large surface area (118). In addition, the easy surface functionalization of CNT allows it to be used as a gene delivery tool (119). For instance, a vaccine against koi herpesvirus (KHV), produced by combining single walled CNT with a plasmid expressing KHV ORF149, enhanced 33.9% protection rate over the naked DNA vaccine (120). However, existing CNT might be toxic to the tissues as it is often not being degraded thoroughly in the host (121). Graphene oxide with a diameter greater than 1 μm, as a potent vaccine adjuvant, strongly attaches to the surface of DCs and can act as a "nano-zipper", thus promoting the aggregation of DC-T cell clusters and providing a stable microenvironment for T cell activation (122). Fullerene molecules are hollow spheres, ellipsoids, or tubes made of carbon atoms (123). Among all fullerene types, C60 is the most widely used in the medical field (124). C60 can serve as a promising vaccine carrier to present MPXV antigenic proteins in the future (125). Quantum dots are carbon nanoparticles less than 10 nm in size (123). Photoluminescence properties of

quantum dots allow them to become imaging-traceable gene nanocarriers (126). In addition, quantum dots have high transfection efficiency, allowing better delivery of DNA into cells (126). In summary, these metal and carbon nanomaterials mentioned above provide options for designing and developing next-generation MPVX specific vaccines that may elicit potent host immune responses in preclinical animal experiments or human trials.

Concluding remarks and future perspectives

Currently, ACAM2000, JYNNEOS, and LC16m8 are available for individuals to receive vaccination against MPXV infection. However, breakthrough infections and side effects suggest that new-generation vaccines require to be developed. We highlight potential immunogens as targets for MPVX vaccines. The functional roles of M1R, H3L, E8L, and A29L proteins in mediating membrane attachment and fusion indicate that vaccines relying on these proteins could produce effective neutralizing antibodies to block viral infection. The membrane proteins M1R, A29L, E8L, and H3L of IMV and envelope proteins B6R and A35R of EEV are all potential targets of neutralizing antibodies (Figure 5). In addition, A29L, B6R, A35R, and M1R (homologs in VACV) react with CD4+ and CD8+ T cells in PBMCs from VACV-vaccinated donors (61). Hence, these proteins are effective and rational targets for MPXV-specific candidate vaccine development. Rational selection of T- and B- epitopes exposed by the MPXV could provide an effective route in developing potent vaccines. Delivery of MPXV immunogen through multiple platforms is imperative to be developed in the future. These vaccines are believed to be more effective and

immunogenic against MPXV than the traditional smallpox vaccine due to their specificity to MPXV. Overall, research on the vaccine targets of MPXV would provide valuable information for developing timely effective MPXV-specific vaccines.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

H.Z. and KW.Y. conceived ideas and supervised the work. Y.W. and H.Z. wrote the initial draft. H.Z. reviewed and edited the manuscript. All authors reviewed and approved the manuscript before submission. Y.W. and KW.Y. contributed equally.

Vaccine	Manufacturer	Stage of approval status	Characteristics	Dosage and route	Refs
ACAM2000	Sanofi Pasteur Biologics Co	In 2007, licensed by FDA for smallpox.	A live plaque-purified VACV derivative of Dryvax.	Single dose/ percutaneous	(33, 34)
IMVAMUNE/ JYNNEOS (the United States)/ IMVANEX (the European Union)/ MVA-BN(Germany)	Bavarian Nordic	In 2019, FDA approves JYNNEOS for prevention of smallpox and mpox in adults. On August 09, 2022, FDA issued an emergency use for mpox.	A third-generation vaccine based on the non-replicating modified vaccinia virus Ankara (MVA) strain	Two doses, 28 days apart /subcutaneous	(36, 127)
LC16m8	Kaketsuken (Kumamoto, Japan)	In 1980, licensed for use by Japanese Regulatory Authorities for smallpox.	An attenuated, replicating smallpox vaccine derived from the Lister strain of vaccinia	Single dose/ percutaneous	(42, 43)

Table 1 Current vaccination resources

Table 2 Potential target protein of MPXV vaccines

Protein name in MPXV	Protein name in VACV	Sequence similarity	Length (aa)	Location	B cell epitopes (MPXV)	Function	Refs
M1R	L1R	98.40%	250	Membranbe on IMV	Residues 69-91: LSAATETYSGLTPEQKAYVPAMF	Mediate virus entry into cells independently of GAGs. Target of neutralizing mAb.	(47, 48, 86)
					Residues 137-155: YGAPGSPTNLEFINTGSSK		
E8L	D8L	94.74%	304	Membranbe on IMV	Residues 43–62: VRINFKGGYISGGFLPNEYV	Attach on the cell surface by binding to CAGs. Target of neutralizing mAb. Activate and mature dendritic cell (DCs). Induce expansion of effector memory T cells. Induce proliferation of T cells.	(51, 54)
					Residues 94–113: VHWNKKKYSSYEEAKKHDDG		
					Residues 204–223: GSSNHEGKPHYITENYRNPY		
H3L	H3L	93.52%	324	Membranbe on IMV	Residues 13-34: VIDRLPSETFPNVHEHINDQKF 231-239: DNAAKYVEH (discontinuous) (VACV)	Attach on the cell surface by binding to CAGs. Activate and mature DCs. Induce expansion of effector memory T cells. Induce proliferation of T cells. Target of neutralizing mAb. Target of cellular immune responses.	(21, 52, 56-58)

A29L	A27L	94.54%	110	Membranbe on IMV	Residues 21-49: TEFFSTKAAKNPETKREAIVKAYGDDNEETLKQ	binding to CAGs. Facilitate the transport of IMV and the process of envelopment that leads to IEV formation. Target of neutralizing mAb.	9, 60, 62, 128)
A35R	A33R	95.03%	181	Envelope on EEV	Residues 97-127: KESCNGLYYQGSCYILHSDYKSFEDAKA NCA	Elicit T cell response. Regulate the interaction of A36 with microtubule motor proteins. Target of neutralizing mAb. Complement dependent. Key to effective production of infectious EEV.	(65, 67, 68, 86)
B6R	B5R	96.53%	317	Component of envelope on EEV	Residues 49-64: DSGYHSLDPNAVCETD Residues 257-263: CVPSNEEEDPVDDGPDDETDLSKLSKD	Elicit T cell response. Key to effective production of infectious EEV. Major target of EEV-neutralizing antibodies. Induce the formation of actin tails. Complement dependent.	(65, 67, 72, 73, 78, 86)

Amino-acid sequence alignment scores between MPXV (ON 563414.3) and VACV (NC 006998.1) antigens were calculated using the Clustal Omega web tool.

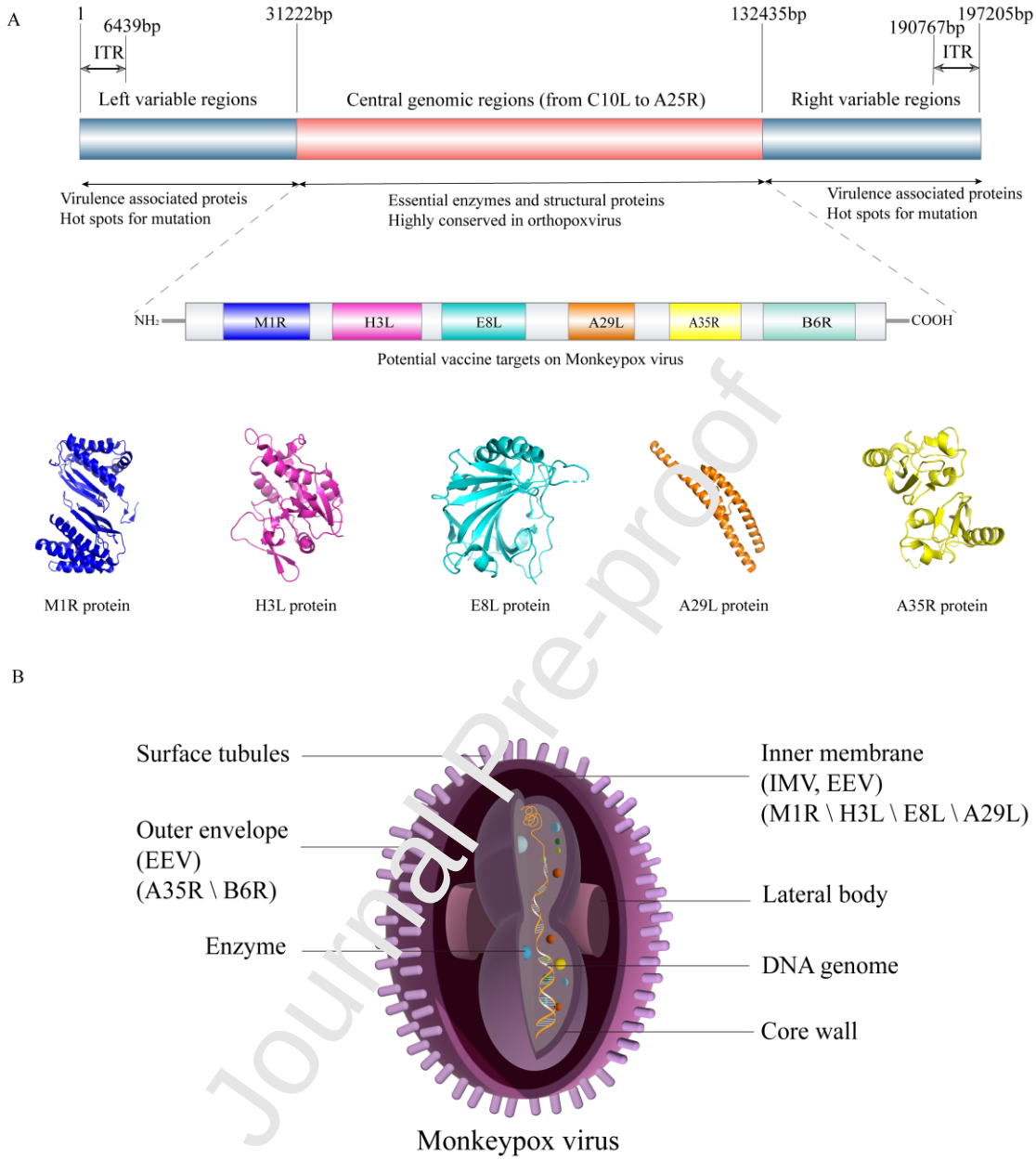

Figure. 1: Genome and structure of mpox virus (MPXV).

(A) The genome of MPXV (ON563414) consists of two variable regions on the left and right and a large conserved central genomic region which is delimited by ORFs C10L and A25R. The left (approximately 25kb in size) and right (approximately 64kb in size) variable regions encode proteins that may be associated with viral virulence and host-range factors and include a same but oppositely oriented 6438 bp sequence, called inverted terminal repeats (ITR) which are the hot spots for mutation. The viral core region

(approximately 101 kb in size), which encodes essential enzymes and structural proteins, is 96.3% identical with the corresponding part of the vaccinia virus (VACV). M1R (PDB ID: 1YPY), E8L (PDB ID: 4E9O), H3L (PDB ID: 5EJ0), A29L (PDB ID: 3VOP), A35R (PDB ID: 3K7B), B6R (no PDB ID available) proteins and their protein structures are represented. (B) MPXV is in the shape of a brick or oval. Two major forms of infectious virions of MPXV are IMV and EEV. The surface tubular proteins of virion are usually in the shape of regular 10 nm long protrusions, beneath which are envelope containing A35R and B6R proteins and membrane containing M1R, H3L, E8L, and A29L proteins. The most significant difference between IMV and EEV is that EEV has an additional envelope containing eight EEV-specific viral proteins. The core of the virus, delimited by the semipermeable shell, contains a large double-stranded linear DNA genome and enzymes. Lateral bodies are located around the core of the virus.

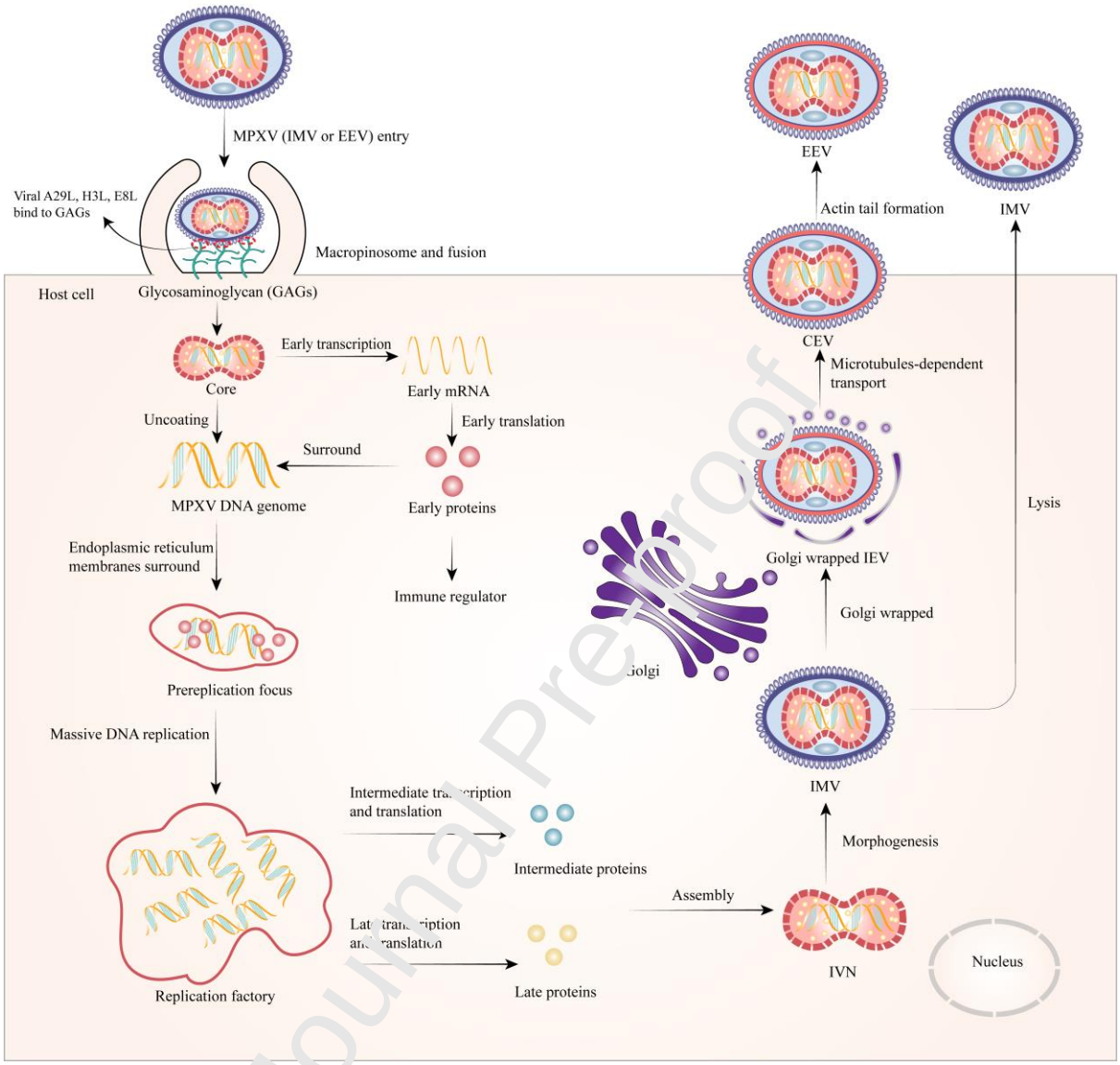

Figure. 2: The life cycle of MPXV.

MPXV surface membrane protein (A29L) attaches to host cell surface glycosaminoglycans (GAGs). Then, the virus activates the host cell macropinocytosis for internalization. After MPXV enters the host cell, the virus begins to perform early, intermediate, and late genetic replication. As DNA synthesis proceeds, the genome is then surrounded by endoplasmic reticulum membranes as well as several early viral proteins to form a pre-replication focus. Discrete prereplication foci grow to form replication factories (RFs) that are surrounded by membranes. The viral genome is wrapped to form immature viruses with nucleoli (IVNs). Then, IVNs mature into intracellular mature virions (IMV), and IMV is encapsulated by a

double membrane from the Golgi apparatus, forming an intracellular enveloped virus (IEV). IEV uses microtubules in Golgi to move from the site of membrane wrapping to the cell surface and fuse with the plasma membrane to form cell associated enveloped viruses (CEV). Then, CEV triggers host cells to form an actin tail that propels the virus out of host cells, and the virion released is called extracellular enveloped virions (EEV).

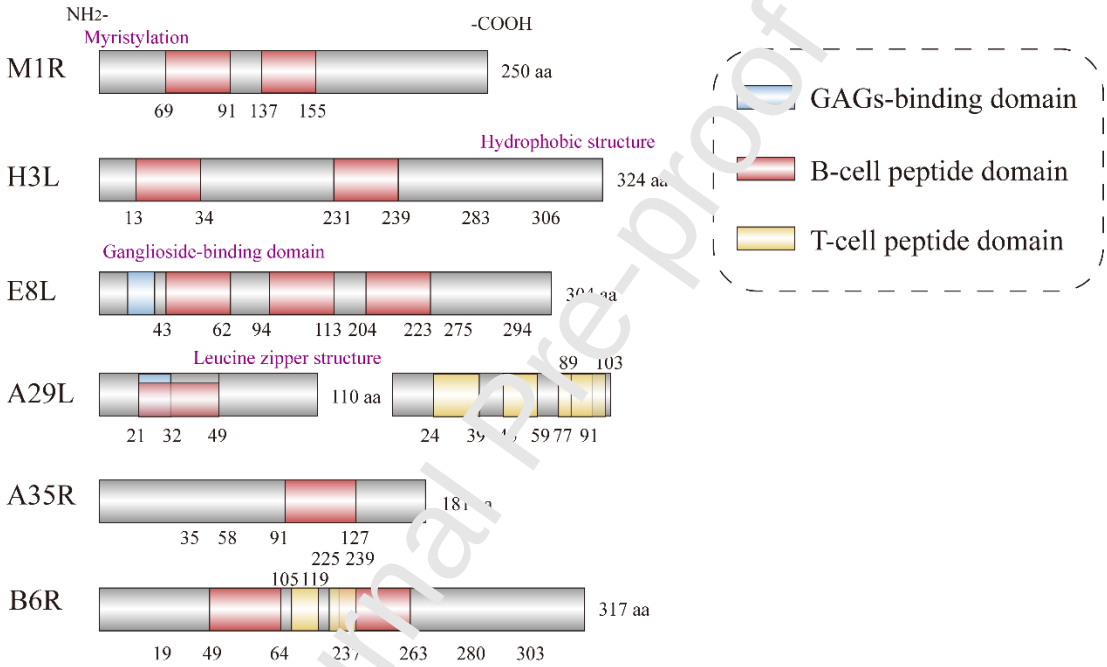

Figure. 3: Amino acid sequences of six vaccine targets

TMHMM 2.0 is used to predict the transmembrane structural domain of amino acid sequences.

GAGs-binding domains, B- and T-cell epitopes domain, and signaling peptides domain on amino acid sequences were shown. The N-terminal and C-terminal specific structural domains of each protein are also indicated.

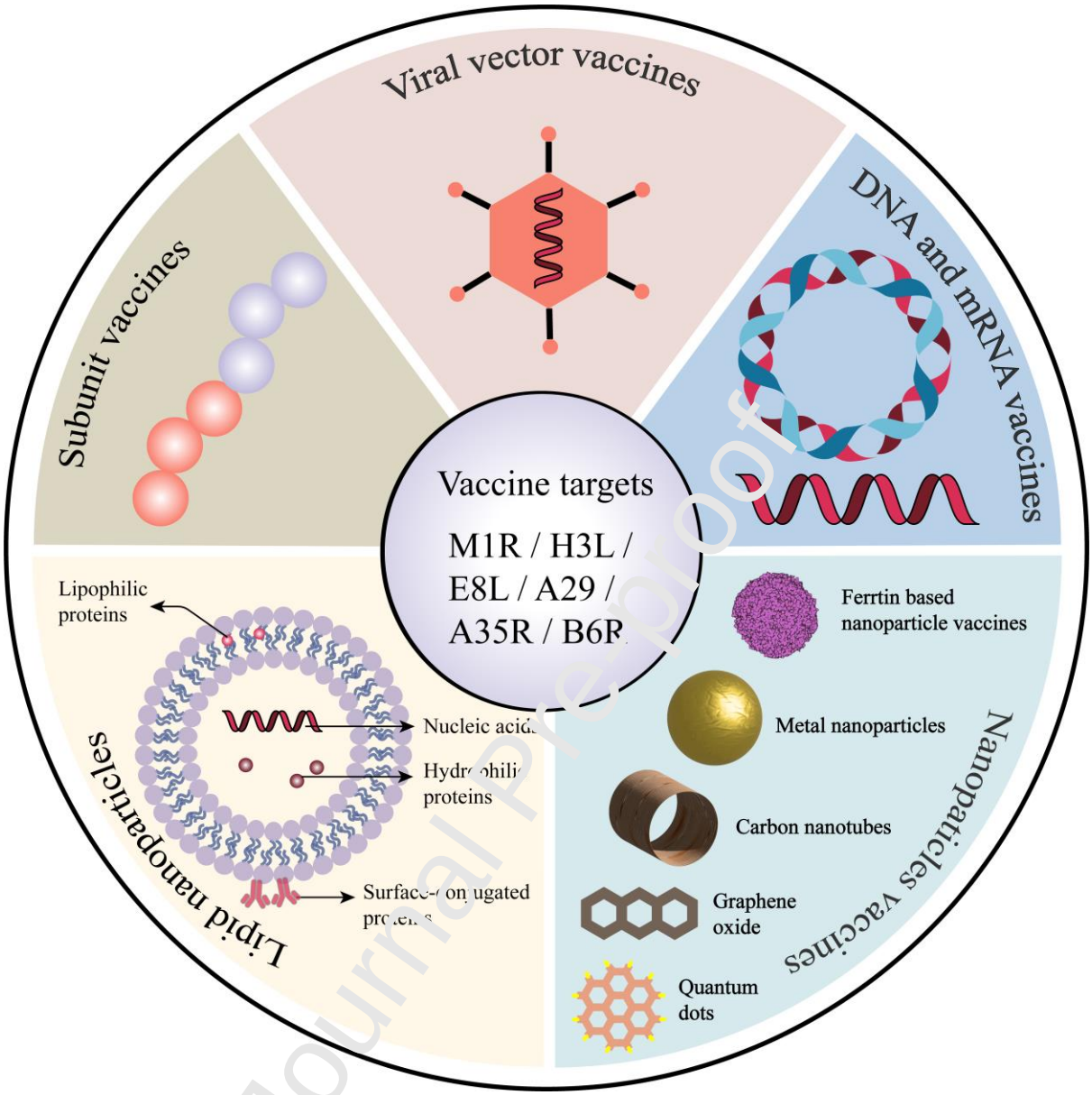

Figure. 4: Different platforms based on the MPXV antigens for MPXV-specific vaccine development.

Multiple platforms such as multiepitope subunit-, mRNA and DNA-, viral vector-, and nanoparticles-based platforms can be used for MPXV-specific vaccines to deliver the target proteins to elicit host robust humoral and cellular immunity.

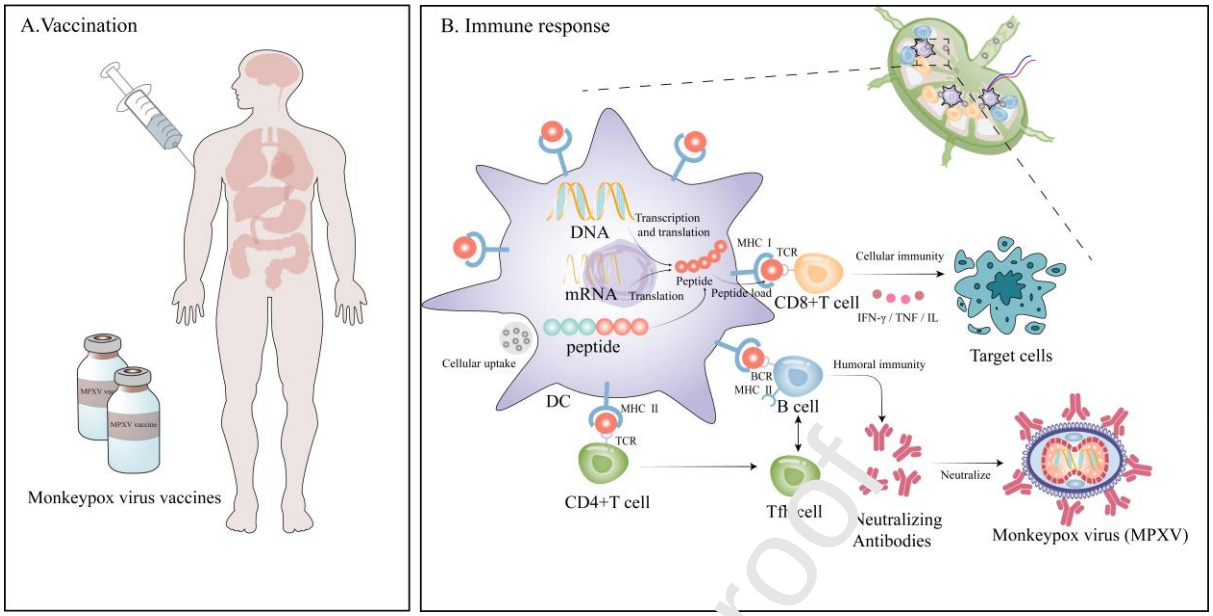

Figure. 5: Immune response to monkeypox virus vaccination

A. Vaccination with monkeypox virus-specific vaccines. B. Dendritic cells (DCs) in the lymph nodes react intracellularly to antigens presented by different types of vaccines via PRRs and load antigenic peptides via MHC. B cells recognize antigens directly and initiate humoral immune responses via BCRs and present antigenic peptide fragments to helper T cells (CD4+ T cell) via MHC class II molecules. The activation of CD4+ T cells by APCs causes them to differentiate into different subtypes, such as T follicular helper cells (Tfh cell), which also help B cells to differentiate into memory B cells and antibody-secreting plasma cells and promote the production of high-affinity antibodies. Cytotoxic T cells (CD8+ T cell) recognize antigenic peptide fragments presented by MHC class I through TCRs and trigger a cellular immune response.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.