REVIEW ARTICLE

A Historical to Future Perspective of Monkeypox Virus and Future Immunotherapeutics

Brent Brown[1]

1 Independent researcher

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Abstract

Monkeypox virus (MPXV) isolation occurred in 1958, with previously non–endemic areas initially announced in 2003, with the second epidemic occurring in 2022. The third announcement occurred in 2024 was confirmed by the World Health Organisation (WHO). The second MPXV outbreak was found to be a lineage derived from clade IIb named the B1 strain; however, between 2023-2024, clade I and clade II MPXV strains are known to have sub–lineages with potential apparence of co–circulation. Historically, the now extinct variola virus (VARV) used to occur in two characterised types (minor / major), with the latter the causal agent of smallpox disease in severity. MPXV was also characterised into two clades (clade I/II), and further lineages with similar differential infection fatality rate (IFR) previously. Existing therapeutics were evaluated since 1796 and are considered further in this review as prophylactic or post–exposure prophylactics. These were investigated through homology of proteins between both MPXV and VARV since the second was eradicated. This review has three objectives. Firstly, an analysis of the epidemiology of the current MPXV outbreak, in context with other poxviruses. The second encompasses historical development of therapeutics to the above two, whilst the third spans potential future cancer therapeutics arising through investigational new drug developmental research since vaccinia virus (VACV) complete genome sequencing occurred in the 1990s.

Corresponding author: Brent Brown, abrownbscmsc@gmail.com

1. Introduction

1.1. Background

During 2024, on the August 13th, an announcement by the Africa Centre of Disease Control was followed on August 14th by the World Health Organisation (WHO). This declaration related to monkeypox virus (MPXV) announced as a public health emergency of international concern (PHEIC), causal of mpox disease. MPXV infections occurred before and after the declared end of the other second 21st century pandemic, severe–acquired–respiratory–coronavirus respiratory syndrome (SARS–CoV–2), causal of coronavirus disease–19 (COVID–19) in 2020 following influenza in 2009. Before the

21st century, respiratory infections caused by viral infections like influenza were characterised. Nevertheless, MPXV is of the *Orthopoxviridae* genus, a member of the family *Poxviridae* known to cause mpox disease characterised by skin lesions. The *Orthopoxviridae* genus includes other orthopoxviruses (OPXVs), like vaccinia virus (VACV), but also the cowpox virus (CPXV), and the variola virus (VARV). The third was causal of smallpox disease, eradicated in 1977, only known to exist in humans; whilst the first two are considered to originate in animals with MPXV initially isolated outside humans able to spread through zoonotic spillover. Therefore, accordingly, at time of writing, one risk analysis classifies SARS–COV–2 as the highest risk of zoonotic spillover[1]. This is followed by two OPXVs, CPXV or MPXV, which ranks MPXV as the 24th most likely virus to present a serious risk to humans. This is defined by 3 host factors (environmental, epidemiology and genetics) together with environmental factors as well as viral factors[1]. The WHO currently classifies MPXV as low–risk.

1.2. Historical perspectives

The earliest records detailing a cousin of MPXV, originate in medical writing, describe a smallpox–like disease in various countries including China, India but also in the writings of the Pharoah Ramzes V in Egypt (< 1150BC). The epidemic spread globally throughout the 6th and 7th centuries across continents[2]. Early origins of the first remedies to smallpox disease are documented from 1796 by Dr. Edward Jenner in the United Kingdom (UK), leading to the smallpox vaccination eradication programme operating for the most part between 1796 and 1975 globally[2]. This occurred through usage of what was then potentially or is considered now as a vaccinia virus (VACV) vector with unknown origins[2]. It was initially in 1938 when Robert Downe was the first person to examine CPXV infection of cells, through examination under the microscope to observe differences in cellular infection then noted as type A (acidophilic) or A–type inclusion (ATI) bodies of infected host cells, later known across other OPXVs[3][4]. At this juncture, the prototype CPXV strain was defined, whilst being studied in infected farmers in the United Kingdom (UK), later denoted as the CPXV British Green (CPXV–BR) strain. Shortly after in 1956, VACV was also first observed under an electron microscope[3][4]. This was followed by the discovery of the structure of DNA in the 1950s, with in 1971 early reports of the genomic characterisation of one of the earlier used strains for smallpox eradication. To this effect, VACV–Wyeth was characterised derived from an isolated seed vial[5]. Subsequently the nomenclature of OPXV proteins evolved derived and defined by the direction (right or left (R/L)) of DNA endonuclease restriction enzyme digests utilised[3][6]. Indeed the protein terminology of VACV was and is confusing, until the OPXV gene (*OPG*) terminology was adopted[7]. Smallpox disease eradication with advancements in technology was confirmed shortly after 1980 by the WHO. The completion of whole–genome and then next generation sequencing (NGS) of many VACV strains has occurred since[8]. Some monitor *in vitro* the reduced plaque formation ability of the VACV extracellular enveloped virus (EEV)[3][8]. However, whilst the VARV genome constructs are not currently available apart from historical analysis, origins remains unknown to this day, whilst existing remnants are understandably held in high security unknown locations with viability unknown or genome–sequence currently (see Supplementary materials)

Decreasing case counts of smallpox disease occurred throughout the pre–1970s epidemics until last recorded fatality in 1975 and 1977 of the then known 2 distinct types of variola (*major*/*minor*) with variable infection fatality rates (IFR) (see

Supplementary Materials)[2]. Around this time MPXV emerged isolated in 1958, designated in the same family of OPXVs. Laboratory isolation initially occurred from cynomolgus macaques (*Macaca fascicularis*) in Copenhagen, followed up in 1970 with the Democratic Republic of Congo (DRC) reporting the first case known to infect human species confirming its zoonotic nature[9]. Frequent outbreaks were documented thereafter with characterisation in both Central and Western Africa countries confirming MPXV. Prairie dogs were also subsequently found to be carriers of MPXV during the 2003 United States of America (USA) MPXV outbreaks[10].

Characterisation in the DRC and surrounding countries occurred before 2022 when two predominant clades of MPXV were defined globally[10]. MPXV clade 1a was confirmed prior to 2014, as a predominant lineage in humans as well as through genome sequencing within the Lunda rope squirrel (*Funisciurus bayonii*) according to nextstrain data reports (Accession number MT724771) (see Supplementary Materials). Clade IIb was also further found to be a predominant clade, causal of the first human outbreak globally of mpox disease in 2022, outside previously known endemic areas of Central and Western Africa[10]. It was further confirmed that clade IIb.B1 was a predominant lineage in 2022-2023[11][12]. Some investigators now use phylogenetic analysis, since changes in the amino–acids (cytosine, thymine, guanine and adenine) and the apolipoprotein B messenger RNA (mRNA) editing catalytic polypeptide–like 3 (APOBEC3) enzymes may hold further clues as to the molecular clock and evolution of MPXV[11][13]. Below is the historical MPXV clade divergence is illustrated.

In 2024, other MPXV sub–lineages were documented, like clade Ib, as research continues. It is notable that current knowledge of MPXV does indicate variability in occurrence between clades, as well as previously documented epidemiological characteristics. For example, the IFR was historically considered to be higher in clade I MPXVs compared to clade II[10]. Many variable factors exist as OPXVs are known in animal reservoirs. Nimaei et al[14] document the array of *Poxviridae* research in animals in recent times further discussed below. Variable factors to detect OPXVs include homologous antigens expressed across this genus utilised for diagnostic antigen detection available globally during the SARS–COV2 pandemic. The WHO to this effect did invite submission of an expression of interest from manufacturers to further evaluate *in vitro* mpox diagnostics in 2024. Currently, only one has been authorised for use indicated for research use only (RUO). To this effect, the Alinity m MPXV assay is a real–time PCR utilised for qualitative analysis of MPXV DNA, manufactured by Abbott, to detect two MPXV antigens (B7R and J2R) using a DNA primer[15]

1.3. Rationale and objectives of this review

In the 21st century, diagnostics and therapeutics have undergone development and analysis, whilst other novel OPXVs, for example the penguin poxvirus (PEPV) was also characterised in animals[16][17]. Each OPXV expresses cellular surface proteins, when processed and presented by a host cell during host infection are called antigens recognised by Fab many of which are homologous to those expressed by other OPXVs of more virulence, like VARV. Presentation of antigens in the VACV vector can be used to harness the immune system through phagocytes also known as professional antigen–presenting cells (APCs) (e.g. dendritic cells (DCs) monocytes and macrophages (Mϕ) to effect the innate and adaptive immune system cells response (B and T) defined by expression of two typers of cluster of differentiation

molecules (CD4/CD8) amongst many other immune cell phenotypes[18][19].

To this effect, VACV, remains arguably the most heavily researched human vaccine vector grown, manufactured and produced *in vitro* by human populations[14]. Three VACV strains may show less antigenic drift (Tian Tan, Mutter, Mulford), and express antigens like a fourth OPXV, the horsepox virus (HPXV). Many have been cultured *in vitro* use in vaccine vectors[8][20]. VACV strains are considered further below as conservation of OPXV antigens may have broader research and diagnostic application potential.

Poxviruses are known to inhabit an array of non–human animal species with or without pathogenicity and can cause skin diseases, exanthems or lesions which are common occurrences known in both human and animal hosts. The WHO currently defines a possible mpox case as potential symptoms presenting within 21 days as any of the following: fever (>38.5°C), myalgia (muscle pain/body aches), headache, back pain, profound weakness or fatigue, not fully explained as varicella zoster virus (chickenpox), measles, scabies, herpes simplex virus, treponema pallidum (syphilis) or other OPXVs (e.g., buffalopox or vaccinia) (see Supplementary materials). Different OPXVs may have different characteristics and utility. For example, fowlpox virus (FWPV) and canarypox virus (CNPV) are could also be of interest as potential vaccine vectors used in commercial flocks. The variety of *poxviridae* existing in nature is illustrated.

To this effect, a phylogenetic analysis was performed recently in 2009, where Carolei et al.[16] analysed a different OPXV, a novel PEPV where viral antigens were compared alongside VACV. Authors concluded that three proteins (G8R, A3L, and H3L) were shared across flocks and could be related to turkeypox virus (TKPV), as well as the pigeonpox virus (PGPV). VACV has been engineered to be used in vaccines approved for use in humans for some years, because of a favorable safety profile, but also through recombinant technology engineering ability to modify specific protein antigens monitored through loss of /8*9function (LOF)[21]. The historical analysis of changeable OPXV gene LOF is therefore relevant to understanding how the human immune system was stimulated to be able to regulate and eradicate VARV in the first instance. Direct relevance to other OPXVs, like MPXV, now remains a priority in 2024, but potentially with other uses in therapeutic interventions. The mechanisms of action of MPXV virion assembly, host restriction factors, pathogenicity, and entry using adhesion factors is described in our comprehensive review paper[10][22]. MPXV pathogenesis is like other well documented OPXVs occurring through assembly of intracellular mature viruses (IMV), utilising host cell machinery. It then forms a double membrane–layered virion as the extracellular enveloped virion (EEV) forms through host cell entry, cellular replication and then exocytosis. Therefore, the protein antigens that can be targets of ongoing evaluation of therapeutics can be considered further[10][22].

The focus of this review will cover current epidemiology of MPXV in 2024, the historical context of therapeutic interventions, but also currently developed vaccines and those undergoing further evaluation. In addition, utilisation of VACV as a cancer therapeutic will be discussed further. These are listed within the National Clinical Trial database (NCT) (n = 349) as observational, interventional or expanded access in conjunction with the phase of clinical trial database as discussed further below.

2. Past, present and future mpox therapeutic development

2.1. Monkeypox virus epidemiology 2022-2024

The most recent WHO epidemiology reports indicate a minimum of 121 countries globally affected have now reported at least 103,048 laboratory–confirmed MPXV infections, with mortality of 229 individuals (an indicative IFR of 0.02%). This occurred from January 2022 (see Supplementary Materials). These were predominantly clade IIb peaking in August 2022 detected in laboratories in mostly Europe and the USA (See Supplementary Materials). The mid–2022 MPXV epidemic peak of infections was dominated by a sub–lineage of clade IIb, denoted as B1 in many countries globally. These countries included the USA (n = 33,191), Brazil (n = 11,212), Spain (n = 8,084), France (n = 4,272), Colombia (n = 4,249), Mexico (n = 4,124), UK (n = 3 952), Peru (n = 3 875), and Germany (n = 3 857) (see Supplementary Materials). Since the 2022 clade IIb.B1 peak in MPXV confirmed laboratory analysed cases, an average of 866 cases per month was indicated of predominantly clade IIb.B1 outside Africa (see Supplementary Materials).

The more recent 2024 African CDC and WHO declarations in relation to MPXV stem from reports where diagnostic polymerase chain reaction (PCR) sampling of affected individuals (n = 241) was available in Africa, and utilised to further detail clade I in the Democratic Republic of Congo (DRC), with clade Ib defined[23]. At this juncture clade 1b began to be documented. Such data is available on nextstrain of both MPXV clade I and clade II (see Supplementary Materials).

To this effect after August 2023, the more recent MPXV clade Ib was indicated by the WHO as a low level of risk in 2024. On 13th September 2024, the African CDC reports indicate now that 5,549 MPXV infections of any clade have been confirmed with 643 deaths reported (an indicative IFR of 2.11%). These occurred across all 5 regions within 15 African Union (AU) member states, which are likely to have been clade Ia/IIb; whilst clade Ib remains under investigation (see Supplementary Materials). Since June 2024, MPXV clade 1b is documented in the Central African Republic (CAR), Burundi, Rwanda and Kenya. Isolated case reports from Sweden, Thailand, Pakistan, Germany and now the UK have also now confirmed through whole–genome sequencing one sequence of clade Ib together with clade IIb.B1 MPXV lineages (see Supplementary Materials).

On July 28th 2024, the African CDC report details the overall IFR of MPXV as 3.9% since January 2022 clade II MPXV emergence. Furthermore, following up on Clade Ib sequencing, the IFR was further detailed to coincide with 78.5% increase in MPXVPCR confirmed cases, with an IFR in 2024 of 3.2% (see Supplementary materials). However, in October 2024 other reports did confirm such difficulty in quantifying the IFR which varied between 1-10% between clade I/II laboratory analysed MPXV infections, whilst the sub–lineage analysis undergoes further analysis[10][24]. For example, in some reports, the IFR was indicated to be 4.9% (384/ 7851 individuals), and therefore this may be consistent with previously documented clade I reports (See Supplementary Materials). Such assessment could be affected by inferred observations during prior smallpox disease observations many years ago also known as two types (*Variola major* and *Variola minor*) with similarly variable IFR[25]. Variable factors now are most likely a combination of MPXV virulence factors, as well as route of transmission together with pre–existing immunity from other OPXVs, availability of timely healthcare, and many other environmental factors which remain difficult to quantify. Currently, few age stratified reports exist detailing

the 2022-2024 MPXV outbreak epidemiology of clade I / II are known to date. However, 41% of confirmed MPXV infections were considered to occur in children under the age of 15, unlike the 2022 outbreak peak of MPXV clade IIb.B1 (See Supplementary Materials). This should be of concern given that the correlate of VARV immunity remains unknown since cessation of smallpox vaccination globally since 1980[26][27].

In November 2024, further recent detail now indicates that the IFR of MPXV could potentially be 0.4% (57/18025 individuals), and this may concur with reports from 2022 of clade IIb.B1 MPXV outbreaks. However, complexities of OPXV infections given the above can only be now outlined with potentially 2602 out of 95328 potential infections (IFR 2.73%) with source of infection unknown, although Burundi has reported an MPXV IFR of 0% (see Supplementary materials).

2.2. Background to current OPXV based therapeutics

Previously developed vaccines utilised to counter initially VARV and MPXV infection are considered as either prophylactic before / or for post–exposure prophylaxis (PEP) and can prevent serious disease against viral infection. High mortality with VARV was observed during smallpox eradication over many centuries. VACV recombinant vectors were developed subsequently and used as biological vaccines due to homologous whole cell expressed proteins (known as antigens). The VACV vector is engineered through serial passage in cell culture, with loss of function (LOF) together with attenuation of both virulence and replication underpinning its utility in research and the clinic known to produce an antibody (Ab) response[28]. Thus, harnessing this method of delivery of viral antigens for presentation to the host immune system is undergoing further investigation and clinical trials. It is known through attenuation of VACV, that this recombinant virus can be prophylactic against many other OPXVs, including MPXV causal of mpox disease and potentially in other pathologies as below[10].

The origins of VACV utilised to inoculate against VARV remain unknown but are most likely originally derived from either HPXV or CPXV. The initial discoveries occurred around 1796, investigated, and then published by a clinician and researcher Dr. Edward Jenner (1749-1823). Such discovery instigated a change in the variolation technique, replaced by the technique of vaccination utilised in many countries including USA (1800), Spain (1803), with Peru and Russia similarly adopting this technique to contain early smallpox–disease outbreaks[29][30]. Isolation and cultivation of VARV occurred during the 1940s on chorio–allantois chick embryos where VACV and VARV were compared when much of this remained unknown before DNA discovery[31]. It was after this when poxviruses were characterised as double–stranded DNA viruses. In 1964, Mayr and Munz continued this development through passaging VACV in chick embryo fibroblast (CEF) *in vitro* whole–cell culture[32].

During 2011, alternative investigations constructed a DNA vaccine named VennVax, and through analysis of promiscuous VACV / VARV epitopes this was outlined further[32]. This investigation utilised transgenic mice where T cell epitopes as well as peripheral blood sera was stimulated by second generation smallpox vaccines[33]. Genome–derived epitope–driven vaccines thus were compared some years after smallpox eradication, to show that through presentation of epitopes through the cell membrane expressed major histocompatibility complex (MHC) type II protein that could be of use. Indeed, homology of 48 out of the 50 human leukocyte antigen (HLA) proteins was observed, but also many (68%)

were identical to early MPXV strains[33]. Thus, these could offer future potential as peptide boosters. Furthermore, recent reports indicate that there are other epitopes that are promiscuous to other OPXVs and have been validated through reverse vaccinology (e.g., bovine popular stomatitis virus, CPXV, ECTV, VACV, VARV)[33].

Utilising artificial intelligence (AI) tools, which include PoxiPred together with feeding poxvirus proteome codes, modelling epitopes across 25 distinct poxviruses can enable predictive modelling of current OPXVs antigens. Thus, harnessing technology operation systems has illustrated this with regards to the adaptive immune system represented by T cells. As a result, it has been quantified there are potentially 15,389 T–cells expressing CD8 (cytotoxic T cells, T_C) able to recognise specific OPXV epitopes together with a further 1,428 epitopes potentially recognised by T cells expressing CD4 (helper T cells, T_H) thus illustrating potential in future constructs of cross–OPXV vaccine vector candidates[34].

Nevertheless, the first HPXV strain was isolated from sick horses in Mongolia in 1976, with limited data surrounding this OPXV[35]. Few reports exist comparing genomes of VACV, VARV and HPXV, apart from a recent 2017 analysis of a H.K. Mulford 1902 smallpox vaccine that did show, through genome analysis, 99.7% homology with HPXV[36]. This anomaly of unknown origins of VACV/HPXV occurred before the discovery of DNA in the 1950s. During 2018, constructs of the larger HPXV genome, denoted in terms of size as 212 kilobases (kb), were created as a synthetic chimera (scHPXV), designed to initially explore the correlate of protection against lethal VACV infection. This scHPXV construct showed lesser virulence *in vivo* like the more recent usage of VACV in modern day research[37]. HPXV is most likely now extinct in the 20th century, or originates with ancestry with VACV as outlined above (see Figure 3). To this effect in 2023, Tonix pharmaceuticals developed TNX–801, which was a recombinant–chimeric HPXV protein made through gene–synthesis in the absence of a cell isolate of either HPXV or VARV[38]. Therefore in 2024, there is an apparence of potential vaccine platforms to counter OPXVs, the homology of the most extensively researched pathogen family (apart from human immunodeficiency virus) lends creedence to there being other viable therapeutic options in future. For example, a single dose of TNX–801 was also found *in vivo* to protect non–human primates (NHP) against MPXV infection similarly to VACV with research ongoing in 2024[38].

2.3. History to current OPXV based therapeutics

The first generation of vaccines against ancestral pathogenic strains of OPXV, like VARV, included Dryvax®, a replication–competent VACV (derived from NYCBH strain), but also the Aventis Pasteur Smallpox Vaccine (APSV)[39]. Second generation vaccines included ACAM2000® developed from VACV (NYCBH strain) seed stock *in vitro*. A Lister clone (LC) based VACV vaccine was also derived from a cell isolate using rabbit kidney cells (RIVM), used during prior smallpox disease eradication. Third generation low–replicating strains derived from the LC clone were also developed through passaging and culturing on chorioallantoic membranes (known as Lister clone 16m8, LC16m8). Whilst recent 3rd generation OPXV vaccine development since 1964 continue to in 1994 by Mahnel and Mayr who confirmed a VACV derivative, modified Vaccinia Ankara (MVA) strain virus concentration standards as $10^{7.5}$ using the 50% tissue culture infectious dose (TCID50) routinely used in drug evaluation[40][41]. At this juncture, cellular immunity was mentioned as around 1 year, with two injections that were weeks apart[40][41]. Subsequently in 1998, a vial of this non–replicating MVA

strain was transferred from the Institute of Molecular Virology in Munich to Bavarian Nordic (BN), who passaged this further (known as MVA–BN®)[42]. In 2003, other MPXV in the USA occurred when further evaluation of the MVA–BN® vaccine comparing with earlier 1st and 2nd generation VACV vaccines (as well as Elstree–RIVM / Elstree–BN). Further indications were that attenuation of the VACV vector (MVA–BN®) could retain immunogenicity against other both MPXV and other OPXVs[42][43].

In the UK and Europe, the MVA–BN based vaccine is distributed under the brand name Imvanex®, originally licensed in 2013, updated in 2022 by the European Medicines Agency (EMA) together with the committee for medicinal products for human use (CHMP). Whilst, in both the USA and Canada, distribution occurs as Jynneos™ (under brand names Imvamune® or Imvanex®). Initially this was authorised for use by the Food and Drug Administration (FDA) agency in 2019 for use in adults over 18 or those at risk of either smallpox or mpox disease (see Supplementary Materials)[44]. The first third generation MVA–BN vaccine (Imvanex®) was pre–approved by the WHO for use on 13th September 2024 during the current MPXV 2024 outbreak (see Supplementary Materials).

Following the 2003 MPXV outbreak in the USA, Kenner et al.[45] produced reports investigating reactogenicity of an earlier utilised VACV based smallpox vaccine in Japan in the 1970s[46]. This VACV derived vaccine was cultured at low temperature (30°C) within primary rabbit kidney (PRK) cells with further clones evaluated in mammalian cell lines (Vero cells), resulting in a VACV Lister Clone 16 (LC16) being evaluated. The resultant *in vitro* derived LC16 cell line was further passaged to produce LC16m8 which was evaluated for growth in mammalian skin, as well as for effects within the central nervous system (CNS) *in vivo.* There was an appearance of less erythematous reactions upon immunisation[46]. Prior to this the Japanese Ministry of Health had formed a Smallpox Vaccine Research Group (SVRG) in 1966. A comparison of various Lister strain clones (e.g. LC16, LC16m8, LC16mO) occurred, together with other like CVI–78. It was then found that the LC16m8 strain could not replicate above a temperature of 40.5^{O}C compared to other strains[47][48]. Whilst LC16 had been used prior to 1975, LC16m8 was licensed in Japan and is produced by KM Biologics[47][48].

The utilised LC16m8 VACV strain was determined to have a deletion in the*B5R* gene and phase I/II clinical trials occurred in the USA subsequently. Now prior to 2011, LC16m8 was compared with other smallpox vaccines. Indeed neutralising Abs (nAbs) were also produced with little reactogenicity[47][49]. It was indicated that deletion of the VACV*B5R* gene was considered to reduce EEV production by OPXV infected host cells[47][49]. LC16m8 has been compared in other reports with MVA–BN[47][48]. The WHO therefore duly pre–qualified the second mpox vaccine, LC16m8, together with MVA–BN for usage within mpox disease outbreaks settings. Early research and clinical usage of LC16m8 had investigated this in both adults as well as children (n>50,000), with little evidence of reactogenicity in the 1970s. Thus the loss of virulence of OPXVs may shape evolution of current evaluated uses as vector vaccines in modern day research as well as clinical settings to minimise potential future threats of other more virulent viruses[7][50][51].

2.4. December 2024 updates on vaccines and diagnostics for mpox

The Health Emergency Preparedness and Response Authority (HERA) from the European Commission committed in August 2024, in a partnership with Bavarian Nordic, to supply 175,420 MPXV vaccines, with the second supplying 40,000

vaccine doses to the African Centre of Disease Control (CDC). The WHO also invited a submission of an expression of interest from manufacturers to provide emergency use authorisation (EUA) to affected populations, but also facilitate regulatory approval in various countries of both medical devices and vaccines (Supplementary Materials). Supply of vaccines is subject to individual country regulatory authority licensing, but also has limitations based on manufacturing, distribution, and quality control as well as supply.

Thus, at time of writing, Bavarian Nordic have indicated that potential manufacture can occur before the end of 2025 of up to 10 million vaccines to counter MPXV infection. Various countries globally hold limited reserves of the second, but more of the third generation of originally used smallpox vaccines, due to previous smallpox epidemics prior to 1980. To this effect reports to date currently indicate that the USA will supply up to 50,000 doses of the FDA licensed vaccine Jyneeos™, which is authorised for use in adults only and specific risk groups (see Supplementary Materials). Additionally, both France and Germany have committed to supplying 100,000 doses, whilst Spain has indicated in the region of 500,000 doses. It is currently estimated that Japan will be providing an undisclosed number (varying from 2-3.5 million doses) of the LC16m8 vaccine manufactured. Following licensing of ACAM2000® by the US FDA, an additional 50,000 doses in late August 2024 was announced by the USA manufacturer Emergent BioSolutions. Therefore, current potential availability is indicated at in the region of 4.5 million vaccine doses globally, largely from existing smallpox stockpiles and newly manufactured vaccines. However, during 2015, intradermal and subcutaneous administration of MVA was indicated to be similarly immunogenic when intradermal administration was then shown to be comparable at 1/5 of the original dose[52]. Such factors would affect availability of global vaccine supply.

With 2023 came further development of currently used diagnostic tools. To this effect a real–time polymerase chain reaction (rtPCR) test specific for clade Ib has been validated with alternative primers utilised for PCR for MPXV clade Ib[53]. Schuele et al.[53] recently indicated that *C3L* was missing in MPXV clade Ib, potentially affecting sensitivity of currently used diagnostic PCR primers. The WHO to this effect have suggested either a nucleic acid amplification test (NAAT), such as real–time (rtPCR), or conventional PCR, together with the MPXV clade being defined through sequencing of either skin or mucosal lesions (see Supplementary materials). There is a paucity of reports documenting immunohistochemical analysis after MPXV infection. Some authors describe MPXV infection of individuals ($n = 20$), together with cells stained using haematoxylin / eosin to observe necrosis around viable epidermal keratinocytes, together with Guarnieri inclusion bodies, within the cytoplasm of enlarged affected keratinocytes. Upon staining with a generic OPXV Ab (rabbit anti–VACV immunoglobulin (Ig)), observations occurred of scattering of dendritic cells (DCs), together with an increased proportion of T cells expressing a cluster of differentiation protein ($CD8^+$) within the epithelial cell dermal layer seen[54]. This would therefore be suggestive that both DCs and T_C cell responses are occurring against the IMV/EEV formed during MPXV infection.

2.5. Current mpox therapeutics

Individuals who had received the smallpox vaccines before 1980 when MPXV arose, indicate that efficacy of the initial smallpox vaccines utilised was 85.1% and 87.1% against secondary infection with MPXV[42]. This data from the 1980s was further evaluated. Observations in two key studies examining reduction of MPXV transmission in both smallpox

immunised as well as non–immunised individuals occurred. Two studies (n = 1578 and n = 2278) of individual cohort groups documented close contacts of those exposed to MPXV to indicate the occurrence of MPXV[42]. This was indicated in immunised and non–immunised individuals to be reduced[42]. Each study respectively showed a 7.4%:1.1%, compared to 7.47%:0.96% reduction respectively of MPXV infection in those immunised with the 1st generation of smallpox vaccines[42]. Therefore this infers 1st generations of smallpox vaccines did have a prophylactic effect on MPX Vas a prophylactic. This prophylactic effect occurs utilising APCs. For example, the cells within the dermal layer are rich in immune cells like both DCs, and Mϕ as well as Langerhans cells that are required to present MPXV antigens to the immune system at primary, secondary as well as tertiary lymphoid structures within anatomical sites[55].

Table 1. 21st century VACV vaccine phase I–IV clinical trials and observational studies (O)

NCT	Vector	Study Objectives	Infection	Phase	Citation / Notes
06549530	MVA–BN®	Evaluate the safety and immune response of MVA–BN in VACV naïve individuals Participants: healthy adults aged 18-50 years (n = 229)	NA	II	Observational, completed October 2023
04639466	COH04S1	COH04S1/synthetic MVA. Primary endpoints: MVA–specific or MPXV cross–reactive binding antibodies and T cells evaluated by ELISA, PRNT and TCID50	mpox	I	[56]
01144637	MVA–BN®	VACV–specific Ab titers measured using a PRNT and ELISA (n=4005).	smallpox	III	[57]
00316589	MVA–BN®	Study to Evaluate Safety and Immunogenicity of MVA–BN® in 18-55-Year-Old (CD4 Counts >200 – 750/µl) n=581)	smallpox	II	[58]
03699124	Freeze–Dried (FD) MVA–BN®	Primary end point:Randomised evaluation FD MVA–BN® and safety and immunogenicity (n = 1129) in healthy adults..	smallpox	III	[59]
05740982	MVA–BN®	Primary end point: mpox PEP: A cluster randomised controlled trial' (n=1500)	mpox	II	CEPI and Canadian Institutes of Health Research funded SMART trial. Ethics and regulatory approved in DRC
jRCTs 031220171	LC16m8	Primary end point: nAb seroconversion rate against MPXV (n=50)	mpox	I	[60]

After 2023, on June 28th 2024, a phase 4 investigational study was completed evaluating MVA–BN as an intervention against MPXV (NCT05734508) in adults (n = 500) after the above (see Supplementary materials). To this effect both MVA–BN and LC16m8 have both met the WHO criteria and safety of the LC16m8 was evaluated (n>50,000) in individuals. MVA-BN is currently consider for those at risk of mpox disease over the age of 12 whilst phase II evaluates

age 2-10. This was a reasonable observation as in 2013

3. Future development for OPXV vaccines platforms

3.1. Factors in MPXV immune responses

Early reports indicated that there were issues passaging VACV *in vitro* as indicated[61]. However, monitoring of plaque formation and neutralisation within infected *in vitro* cultured cells like baby hamster kidney fibroblast cells lines (e.g., BHK21), together with deletion of cellular growth genes (e.g. *F13L*) has evolved and developed a long way since 1980[61]. Selective insertion of other genes to synthesise a green fluorescent protein (GFP) for visualisation allows insight into such methods of recombinant technology engineering[61]. Other research groups similarly removed alternative VACV genes (e.g., *K17L*) *in vivo* from VACV–WR strains[62]. This was also seen to contribute towards lesser VACV pathogenicity via intradermal or intranasal routes *in vivo*[62].

The essence of a viral vector used as vaccines is that antibodies (Abs) should be produced against the VACV host cell expressed antigen to evoke not only host production of IgG by B cells in response to infection, but also a necessary Tc cell response utilising cytokinest stimulating growth factors (CSF). To this effect, deletion from MVA of another three VACV genes (*A44L*, *A46R* and *C12L*) further elucidated that both T_H lymphocytes as well as Tc cell phenotypes could still be evoked[63]. The migration of T cells through the lymphoid vascular system was shown significantly to secrete type II IFN–γ, IL–12, together with both IL–1β and type I IFN–β[63]. Each of these cytokines is considered to be crucial to T cell function, DC maturation and Mϕ cell-cycle regulation[64].

Few reports document antibodies produced against VACV in the general human or animal population globally remaining becoming known in the 21st century, because of early cross-specificity of OPXV reagents. VARV was only known in humans then with scientific technology that did not exist to stratify such details now. For example, 21 years ago, after the USA MPXV outbreak, 2003-2019 reports quantified seroprevalence of OPXV neutralizing IgG antibodies across continents. OPXV antibodies were compared and quantified between Mali (17.25%), France (1-10%), Bolivia (<1%), Laos (<5%)[65]. Such figures are notable, given that OPXVs exist in nature as outlined above. Subsequently during 2018, Costa et al.[66] utilised the plaque reduction neutralization test (PRNT) to evaluate OPXV antibodies. It was then indicated that 19-22.8% of dogs were seropositive for OPXVs in this study[66]. Further to this in 2019, OPXV antibodies were quantified during a VACV outbreak in humans, to indicate IgG antibodies produced in non–smallpox immunised individuals occurred in 31% of individuals in the farming industry during a VACV outbreak[67]. As recently as 2023, Ilchmann et al.[68] further concluded that either one or two doses of the MVA–BN vaccine could induce stable B cell memory responses over 2 years (n = 753) (NCT00316524 and NCT00686582). Similarly other systematic reviews examined the immunogenicity during randomised control trials (RCT)[69]. Whilst in 2024, in Kenya, in an age–group analysis (age 20-55,n = 99) of OPXV infections it was confirmed that IgG was produced against OPXVs with an average of 36.9% increasing to 85.8% in individuals testing positive for HIV[70]. Notably, this group detailed and utilised OPXV antigens in an enzyme–linked immunosorbent assay (ELISA), which utilises monoclonal antibodies (mAb) designed to be specific. It was then illustrated

that there was 198% cross–reactivity for MPXV A35R (as compared to VACV A33R), compared to 43.4% for MPXV E8L (as compared to VACV D9)[70]. Such variability in OPXV antigen specificity, given the rarity of other OPXV infections in humans therefore is useful in choice of antigens by both diagnostic and therapeutic manufacturers.

3.2. Future prospects for MPXV vaccines in development

Alternative vaccine manufacturing platforms are now advancing other methods of expressing VACV antigens in 2023. An initial mRNA vaccine (mRNA–1769), part of the mPower trial has enrolled individuals (n = 351) participating in randomised phase I and phase II clinical trials (NCT05995275), with the aim to evaluate this method of delivery to target MPXV prophylaxis. This method utilises the lipid nanoparticle (LNP), together with OPXV antigens with knowledge derived from research of all prior OPXV protein antigens. Zhang et al.[71] also recently evaluated a second potential mRNA vaccine candidate (MPXVac–0097), beginning development *in vitro*, utilising LNPs encoding five specific VACV antigens (M1R, E8L, A29L, A35R, as well as B6R). This report indicates that mRNA vaccine constructs expressing MPXV antigens evoke a specific T_H1 cell response[71]. Additionally, the authors measured leukocytes to find type II IFN–γ secreted alongside both tumour necrosis factor alpha (TNF–α), together with interleukin 2 (IL–2) in the spleen alongside a Tc cell response (secreting both type II IFN–γ / TNF–α)[71]. Furthermore, other antigens expressed by VACV (A27, L1, A33, and B5) are of consideration and could be cross–prophylactic during OPXV pathogenesis. For example, such proteins are involved with the adsorption, binding, and intercellular transmission of OPXV infected cells. Both A27 protein and the L1 protein on IMVs are generally considered to mediate the attachment and binding of VACV to cells[72][73]. Whilst the other two VACV A33/B5 protein are expressed by EEVs[74]. To this effect a third mRNA vaccine construct also investigated OPXV antigens expressed across three OPXVs (VACV, VARV and MPXV), to choose five specific OPXV antigens (A29, A30, A35, B6, and M1)[75]. In 2024, other pre–clinical investigations indeed have also begun*in vivo* (BNT166a/BNT166c) analysing MPXV antigens (A35, B6, M1, H3) with one (BNT166a) progressing into a BioNTech–SE sponsored clinical trial (NCT05988203).

Such reports are only now comparing the three relevant clades of OPXVs (VACV, MPXV and VARV). These reports all seem to agree, indicating through x–ray crystallography analysis of the protein structure formation to show that L1 (M1) proteins are composed of 250 amino–acid residues which are crucial to OPXV IMV formation[76][77]. It was duly noted that removal of this L1 (M1) protein in VACV could abrogate its ability to mature and form an IMV[77].For example, Su et al. in 2007[77] did show that neutralising antibodies (nAb) could be generated in response to a VACV vector expressing L1. Indeed comparison with a baculovirus vector expressing L1, glycosylation of antibodies could affect neutralising ability of this protein. At this juncture, bivalency of mAb–7D11 was shown and to be indicated to bind to a specific epitope of the L1 protein with variable avidity. It is described by others as 99% homologous across three OPXV clades[77][78].

3.3. VACV as an OPXV based viral-vaccine vector

VACV is demonstrated to stimulate the innate immune system, as defined by B cells to produce IgG antibodies (which also occur in 4 types), both T cells and natural killer (NK) cell phenotypes are equally important parts of the adaptive

immune system. For example, NK cells were investigated during 2013. Summary reports indicate in NHP that MPXV substantially stimulates NK cell expansion and proliferation with decreased NK cell degranulation, but with production of both type II IFN and TNF[79]. Hickman et al[80] in 2015 further showed that VACV administered epicutaneously was dependant on CXCR3 in ablation studies, but could upregulate two known ligands, CXCL9 and CXCL10, and be dependent on Tc cells for viral clearance. This cellular mediated adaptive immune response is therefore a likely major part of immune responses to OPXVs, regardless of antibody mediated responses. To this effect, below VACV has undergone further evaluation to counter other viral infections as detailed below in various national clinical trials (NCT) (see Table 2).

Table 2. Other clinical trials investigating VACV safety and immunogenicity

NCT	Vector	Study Details	Target	Phase	Citation
04119440	MVA expressing MERS–CoV spike (S) GP	Primary endpoints: the safety and immunogenicity by assessing MVA–MERS–S Ab responses through neutralisation assays and ELISA (n = 244)	MERS	Ib	[81]
02509494	MVA–BN– Filo–vector and human adenovirus serotype 26 (Ad26.ZEBOV) encoding EBOV-GP	Primary endpoints: the safety and immunogenicity of two candidate vaccines in a 2–dose heterologous regimen. Ad26.ZEBOV and MVA–BN–Filo	EBOV, SUDV, MARV	III	[82]
2661464	MVA–BN–Filo and Ad26 expressing EBOV-GP (Ad26.ZEBOV)	Primary endpoints: assessing long–term safety and collecting serious adverse events (SAEs), as well as pregnancy outcomes among recipients	EBOV	III	[83]
03307915.	Ad26.Mos4.HIV, MVA–Mosaic	Primary end points: safety, immunogenicity and HIV Env–specific functional Ab responses with T cell responses (week 0 and week 40).	HIV	I	[84]
2315703	Ad26.Mos.HIV, MVA–Mosaic	Primary end points: HIV envelope binding Ab responses, functionality of vaccine–induced Ab responses (ADCP, nAbs), and T–cell responses (IFN– γ ELISpot).	HIV	I/IIa	[85]
04186000	Prime–boost regimens using Ad26.ZEBOV and MVA–BN–Filo	Primary end points:the immunogenicity after vaccination, including measuring Ab levels using the Filovirus Animal Non–Clinical Group (FANG) ELISA assay.	EBOV	I/II	[86]
02876328	Evaluation of Ad26.ZEBOV, MVA–BN–Filo and rVSVΔG–ZEBOV–GP	Primary end points: Safety and evaluate long–term memory T–cell responses induced by three vaccine regimens: Ad26–MVA, rVSV, and rVSV–booster	EBOV	II	[87]

MERS: middle-east respiratory syndrome: rVSV: replication-competent, recombinant vesicular stomatitis virus; GP: glycoprotein; ZEBOV: Zaire; Filo: filovirus; ADCP: antibody-dependent cell phagocytosis.

3.4 VACV as a future potential immunotherapeutic

OPXV infection, for example with MPXV, may instigate early changes in expression by immune cells of either cluster of differentiation (CD) molecules as well as interleukins (IL) (e.g. interferon (IFN), as well as chemokines only characterised in the 20th century whilst retaining lack of virulence chemokine[88]. For example, it was observed that MPXV induced a transient early reduced expression by NK cells of CXCR3, CCR7, and CCR6 with later increases following that were CXCR3 and CCR5[79]. CCR7 is considered relevant to naïve T cell migration, whilst CCR5 is considered relevant to trafficking both effector T cells and memory T cells, with the first chemokine CXCR3 considered to have three key ligands

(CXCL9, CXCL10, CXCL10) on a variety of immune cell phenotypes. CXCR3 function remains largely unknown because of its various isoforms[89][90][91]. Its expression in conjunction with other immunotherapeutics may be able to modulate the accumulation of T_C cells regulating tumour growth through tumour–associated antigens (TAAs) expression recognised and presented by different types of DC during malignancy[92]. To this effect, many investigations are ongoing investigating VACV engineered as below to express the brachyury antigen which is considered to be expressed during epithelial–mesenchymal transition (EMT) (see Table 3 below).

Table 3. Ongoing evaluation and investigating VACV as a potential cancer therapeutic

NCT	Vector	Details	Cancer Type	Phase	Citation / Details
4607850	ChAdOx1–HPV and MVA–HPV (VTP–200)	Primary endpoints: safety. Secondary endpoints efficacy / dosage to assess T cells using cytobrush, Cohort age 25–55, (n = 99)	HPV–related low–grade lesions (CIN1)	I/IIb	Conducted by Barinthus Biotherapeutics (formerly Vaccitech).
3438344	Multi–antigen CMV–MVA	Primary endpoints: CMV reactivation, secondary endpoints: CMV–specific T–cell immunity, NK cell function	Haematologic malignancies (e.g., ALL, AML, CML)	II	City of Hope Medical Center. Estimated completion 1st December 2021.
3610581	Adenovirus serotype 26 (Ad26) and MVA	Primary endpoints: Safety, reactogenicity and immunogenicity of adenovirus HPV–specific CD4+ and CD8+ T–cell responses (IFN–γ, IL–2, TNF–α) (n = 66)	HPV16/18 carcinoma	I/IIa	Conducted by Janssen and Bavarian Nordic. Estimated completion December 2022.
3349983	MVA–BN–Brachyury and FPV–Brachyury	Expression of brachyury and T–cell costimulatory molecules, aiming to enhance the immune response in solid tumours.	Metastatic or unresectable advanced malignant solid tumours, (e.g. lung, breast, ovarian, prostate etc)	I	[93]
2179515	MVA–brachyury–TRICOM	Brachyury–specific T–cell responses (CD4+ and CD8+), increase in immune activation via co–stimulatory molecules (B7.1, ICAM–1, LFA–3)	Lung, breast, prostate, ovarian, colorectal, pancreatic cancers, and chordoma	I	[94]
1256853	MVA–EBNA1/LMP2	T–cell responses to MHC class I and II–restricted epitopes within EBNA1 and:, changes in EBV genome levels in plasma	EBV+ nasopharyngeal carcinoma (NPC)		[95]

ChAdOx1: chimpanzee adenovirus vector developed by Oxford University: CMV HPV: human papillomavirus; CIN1: cervical intraepithelial neoplasia: CMV: cytomegolavirus; EBV: Epstein Barr virus: EBNA1: estein–barr nuclear antigen 1; B7.-1: costimulatory ligand, ICAM–1: intercellular cell adhesion molecule-1, LFA–3: leukocyte function associated antigen 3 LMP2: Latent membrane protein 2.

For example, some VACV constructs denoted as derived from the VACV–Copenhagen strain (TG6002) were engineered to lack a thymidine kinase (*TK*) gene, as well as a ribonucleotide reductase (*RR*) gene, but can still replicate only in tumours expressing high amounts of TK and RR proteins[96]. This ability is being examined in different cancer types where treatment options remain limited, or example in other cancer types like malignant pleural mesothelioma. Other options have entered phase I/II clinical trials for glioblastoma and gastrointestinal cancer (NCT03294486, NCT03724071, NCT04194034). Recently Delaunay et al.[97] have investigated insertion of GFP to monitor VACV viral replication within tumours effectively allowing further compartmentalisation into how VACV could be extended beyond its original known usage. To this effect Vasileva et al.[98] *in vitro* utilised a VACV construct derived from the LC strain with deletion of both *TK* and viral growth factor (*vgf*) genes, replacing these with human immune cell growth factors (granulocyte– Mϕ, GM–CSF), together with an oncotoxic protein (lactaptin) inserted into the VACV genome[98]. Investigational biodistribution studies are ongoing to determine blood–brain barrier distribution[98]. Whilst, other researchers have utilised VACV constructs expressing cytokine fusion proteins like IL15–Rα. Similarity of IL–15 with other common γ–chain–sharing cytokines (IL–2, IL–7, and IL–21) can affect checkpoint proteins, like programmed death–1 (PD1), together with its ligand (PD–L1), which in combination with anti PD–1 checkpoint inhibitors have shown promise in initial colorectal and ovarian cancer models[99]. It was noted that this activity was selective for tumour targeting by T_C cells expressing CD8, rather than NK or T_H cells by the authors[99]. For reasons outlined above OPXV suppression of NK cell function may be a factor contributing to this. Whilst other ligands of CXCR3, like CXCL11 have been synthesised and expressed by VACV, early reports investigate suggest this may also enhance efficacy of adoptive T cell therapy[100].

4. Limitations

Firstly, above is subject to approval by individual regulatory authority jurisdiction, whilst import and export of manufactured products requires validation and quality control, laboratory training, and clinical diagnostic facilities. Manufacturing chemicals for immunisation relies on a number of challenges and procedures throughout the development and supply process. This process is defined during production through good laboratory practice (GLP), good manufacturing process guidelines (GMP). Obtaining manufacturing authorisation through individual regulatory authority body submission, like the FDA or European Medicines Agency or the Japanese Pharmaceuticals and Medical Devices Agency is ongoing. Investigating potential route of administration of vaccine antigen and also vector is ongoing. Such challenges include evaluation by manufacturers of route of administration prior, for example, mucosal–administered or parenteral injection (e.g. intramuscular, subcutaneous, or intradermal routes). Full genome sequencing of other VACV–WR strains was only recently completed in the last decade (2018)[101]. Whilst in 2024, knowledge of the OPXV palisade core contained is not fully understood, with crystallisation of other OPXV proteins. for example, A10 trimers only just starting to be emerge[102][103].

5. Discussion

To date, as seen in figure 1-4, together with tables 1-2, the 3rd generation VACV based vaccine vectors developed are of utility to counter mpox disease and have evolved considerably. Many VACV vectors researched *in vitro* were passaged in whole–cell culture during smallpox eradication and are be of use countering other pathogens like EBOV, HIV, as well as cancer. As recently as 2002, direct comparisons of both MVA–BN and LC16m8 occurred thereafter. The second was also derived from a defective VACV–LC strain utilised in the 197s0 eradication of the first VARV eradicated in all human age–groups in Japan[104]. Nevertheless, such methods extend beyond the original VACV derivatives with other vaccine technology platforms available[105]. However, translating the research into producing vaccine supplies is affected by national priorities and reliant upon lengthy production processes and safety evaluation.

Many individual viral antigens and facilities for further development were unknown in the 20th century when characterisation of other viruses like HIV occurred during the 1980s. Importantly immune cells remain characterised by CD molecule and are reliant on the individual MHC haplotype, as well as chemokine proteins characterised further in the 21st century[106][107][108]. Many variable factors affect individual host immune responses that also include diversity of immunoglobulins[109]. Since 2015 CD workshops, OPXVs are known to contain cytokine modulating proteins and to this effect Toll-like receptors are a recent discovery in this field as research continues[110] Tutorial on this here[111]

Tissue–resident Mϕ are most likely the crucial immune cells able to restrain OPXV infection whilst evoking host B and T cell responses[112]. Whilst any OPXV outbreak may be of concern in humans, other alternatives like development of alternative mucosal–administered vaccines could be useful, only just beginning post COVID–19 pandemic[113]. Mucosal options in 2022 suggest that MVA L1being evaluated *in vivo* as an intra–nasal COVID–19 candidate, with initial reports suggesting that an IgA response is produced[113][114]. Future developments will be of relevance to the scientific community.

6. Conclusion

Potential cross–OPXV therapeutics are discussed above, whilst much remains unknown given technological advances in the 21st century. Many of these could have the effect of providing protection against VACV, MPXV and also historical VARV, causal and investigated during and after smallpox disease eradication which have unique proteins antigens expressed. Smallpox disease was only known to affect humans when it was eradicated with the last known case in Somalia in 1977. The original research technology investigating OPXV antigens was unavailable in the early 20th century, but is outlined above. It wasn't until 2007 when Su et al. identified the structure of the VACV L1 (M1) protein to be an antibody target of through structural studies being. a key milestone in OPXV research[77][78].

Evolution of technology systems in the last three decades has occurred and therefore this report serves as an overview, throughout the development of OPXV based therapeutics. Nevertheless, further interventions are required from heads of state, in partnership with public health authorities, as well as partner organisations like the WHO, Coalition for Epidemic Preparedness Innovations (CEPI) and the Global Alliance for Vaccines and Immunization (GAVI), with individual country authorities in order to ensure equitable and timely access. Whilst the origins of VARV remains and is likely to remain

unknown, it is however now possible to see the mechanisms of how eradication of smallpox disease may have occurred, whilst other authors have examined phylogenetic diversity of VARV in relics that may hold further clues in the future[1][10][115][116]. Contact tracing, infection control, and essential resources to endemic regions remains important. The longer–term safety profile of VACV derivatives that early investigations outlined extend into future immunotherapy investigations, like the Brachyury antigen in combination with the above with potential future options as a cancer therapeutic subject to safety studies with further investigations required[117].

Supplementary Materials

The supplementary material for this article is available at:

- October 13th mpox WHO report
- Nextstrain / mpox / all–clades
- 2022-24 Mpox Outbreak: Global Trends
- Bavarian Nordic (bavarian–nordic.com)
- Nextstrain mpox all–clades
- MPox–Situation–in–Africa.pdf (africacdc.org)
- EMA recommends approval of Imvanex for the prevention of mpox
- MVA–BN vaccine in Monkeypox – Clinical Trials Registry – ICH GCP
- Mpox outbreaks in Africa constitute a public health emergency of continental security
- Weekly epidemiological WHO position paper 23rd August
- 9789240076464–eng.pdf (who.int)
- Smallpox/Monkeypox Vaccine CDC Information Statement
- Monkeypox virus clinical trials
- HERA Statement August 2024
- USA Response to Clade I mpox outbreak9789240095519–eng.pdf (who.int)
- mPower mRNA mpox vaccine Clinical Phase 1/2 Trial
- Countries donating mpox vaccines
- Emergent ACAM2000 donation
- DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
- Seegene mpox PCR development
- June 2024 WHO Democratic Republic of Congo Update on mpox
- UK NHS Guidance on mpox
- Vaccine platform tracker 2022
- Smallpox and its eradication / F. Fenner... [et al.] (who.int)1988
- MonkeyPox Virus Typing Tool (genomedetective.com)
- Joint ECDC–WHO Regional Office for Europe Mpox Surveillance Bulletin (europa.eu)

- MPX Reporting Protocol (europa.eu)
- Viral strains derived from the vacv–107 and uses thereof (WO2010031837A1) Google Patents
- Mpox around the World: Current Situation | Mpox | CDC
- Bavarian Nordic (bavarian-nordic.com)

Statements and Declarations

Author contributions

BB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing–original draft, review & editing, Validation, Visualization.

Conflicts of interest

The author(s) declare no conflicts of interest.

Consent to participate

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Availability of data and materials

Requests for accessing the datasets could be directed to the corresponding author (info@biochem123.org) or Supplementary materials.

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