WHO Advisory Committee on Variola Virus Research

Report of the twenty-fifth meeting
Geneva, Switzerland
25–26 October 2023
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# Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>ACVVR</td>
<td>Advisory Committee on Variola Virus Research</td>
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<td>CDC</td>
<td>United States Centers for Disease Control and Prevention</td>
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<td>COVID-19</td>
<td>coronavirus disease 2019</td>
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<td>EAP</td>
<td>Expanded Access Protocol</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EMA</td>
<td>European Medicines Agency</td>
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<td>EU</td>
<td>European Union</td>
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<td>EV</td>
<td>enveloped virion</td>
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<td>US FDA</td>
<td>United States Food and Drug Administration</td>
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<td>HERA</td>
<td>Health Emergency Preparedness and Response Authority (EU)</td>
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<td>IHR</td>
<td>International Health Regulations</td>
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<td>IMV</td>
<td>intracellular mature virion</td>
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<td>IND</td>
<td>investigational new drug</td>
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<td>LAMP</td>
<td>loop mediated isothermal amplification</td>
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<td>LN2</td>
<td>liquid nitrogen</td>
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<td>mAb</td>
<td>monoclonal antibody</td>
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<td>MOH</td>
<td>Ministry of Health</td>
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<td>mpox</td>
<td>monkeypox</td>
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<td>MPXV</td>
<td>monkeypox virus</td>
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<td>MVA-BN</td>
<td>Modified vaccinia Ankara vaccine - Bavarian Nordic</td>
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<td>NIOCH</td>
<td>Novosibirsk Institute of Organic Chemistry</td>
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<td>OPXV</td>
<td>orthopoxviruses</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PFU</td>
<td>plaque-forming unit</td>
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<td>PHEIC</td>
<td>Public Health Emergency of International Concern</td>
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<td>PRNT</td>
<td>plaque reduction neutralization test</td>
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<td>RCT</td>
<td>randomized controlled clinical trial</td>
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<td>SAGE</td>
<td>WHO Strategic Advisory Group of Experts on Immunization</td>
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<td>SI</td>
<td>selectivity index</td>
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<td>VARV</td>
<td>Variola virus</td>
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<tr>
<td>VECTOR</td>
<td>Federal Budgetary Research Institution - State Research Center for Virology and Biotechnology</td>
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<td>WHA</td>
<td>World Health Assembly</td>
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<td>WHO</td>
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Executive summary

The World Health Organization (WHO) Advisory Committee on Variola Virus Research (ACVVR), hereafter referred to as the Committee, held its twenty-fifth meeting on 25-26 October 2023. Ahead of the 77th World Health Assembly in May 2024, this report presents progress to date on the use of live variola virus for development of medical countermeasures for smallpox, a research plan for 2024, and other recommendations offered by the Committee.

Variola virus repositories

The Committee received reports on the variola virus collections held by the WHO Collaborating Centre repositories at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, the United States of America (USA) and at the Federal Budgetary Research Institution – State Research Center of Virology and Biotechnology (VECTOR), Federal Service for Surveillance on Consumer Rights Protection and Human Well-being (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk Oblast, Russian Federation.

Research update

The Committee received reports on progress of approved research using variola virus and presented new proposals. CDC has completed sequencing of all variola virus strains, while at VECTOR, forty-two isolates remained to be sequenced.

Antiviral agents

There are three compounds approved for the treatment of smallpox. Oral tecovirimat is approved for treatment of smallpox by the regulatory authorities in Canada, the European Union (where it is also approved for treatment of mpox), the United Kingdom of Great Britain and Northern Ireland, and the United States of America (where the intravenous formulation is also approved). The rare occurrence of viral resistance to tecovirimat during the global mpox outbreak suggests additional antivirals will be needed. Oral brincidofovir is approved in the USA in the form of tablets for treatment of smallpox in adults and an oral suspension for children. The antiviral agent NIOCH-14 is licensed in the Russian Federation for treatment of smallpox, mpox, and cowpox. VECTOR reported studies of 23 additional compounds with activity against variola virus. CDC proposed continuing study of a humanized mouse model for assessing smallpox therapeutics. Both collaborating centres continue to explore development of monoclonal antibodies for smallpox.

Vaccines

There are 2nd, 3rd, and 4th generation vaccines approved for prevention of smallpox, three of which are also approved for mpox. Vaccine effectiveness against mpox of MVA-BN in several studies during the global outbreak ranged from 66%-86% for two doses, with lower effectiveness noted in persons who reported immune suppression. The regulatory indications for the vaccine in different jurisdictions include prevention of smallpox, mpox and other orthopoxvirus infections. In the Democratic Republic of the Congo, the vaccine has shown an excellent safety profile in health workers and a booster dose study is underway. The LC16 vaccine is approved and in use in Japan for prevention of mpox. In the Russian Federation, the fourth-generation attenuated vaccinia vaccine OrthopoxVac demonstrated lower reactogenicity while retaining immunogenic properties and was licensed in 2022 for prevention of smallpox, mpox, and other orthopoxviruses. CDC presented a new proposal to characterize an orthopoxvirus mRNA vaccine.
Diagnostics

Four kits for detection and differentiation of pathogenic orthopoxviruses developed at VECTOR were licensed in 2017 and 2022. CDC continues to improve nucleic acid- and protein-based rapid diagnostic tests and has developed additional collaborations for diagnostics. Work continues on novel technologies for orthopoxvirus diagnostics using Loop Mediated Isothermal Amplification (LAMP) which showed promising results.

Conclusion

The Committee noted that with the approval of a third antiviral agent and a fourth generation vaccine, the original objectives of the research programme endorsed by the World Health Assembly (WHA) were being met. Countermeasures developed for smallpox preparedness have been key in responding to the global mpox outbreak, illustrating the continuing potential public health benefit of the variola virus research programme. Nonetheless, signals of resistance to tecovirimat in a few cases of mpox were concerning and the Committee noted that continued development of therapeutics with different mechanisms of action is warranted. The Committee noted that the mpox outbreak continued to inspire development of rapid, point-of-care diagnostics and reaffirmed the importance of continuing efforts to develop and validate protein-based orthopoxvirus assays. The Committee also noted that consideration should be given replenishing WHO emergency vaccine reserves.
1. Meeting proceedings

The twenty-fifth meeting of the World Health Organization (WHO) Advisory Committee on Variola Virus Research (ACVVR, hereafter referred to as the Committee) was held at WHO headquarters, Geneva, Switzerland on 25–26 October 2023 by video conference. The meeting was chaired by Dr David Ulaeto. The agenda is included as Annex 1 and the list of participants as Annex 2. The approval status of research proposals from the WHO collaborating centres is presented in Annex 3. Abstracts summarizing progress on WHO approved research projects are in Annex 4 along with summaries of presentations from invited guests.

All ACVVR members, temporary advisors and invited presenters participating in the meeting completed and submitted to the WHO Smallpox Secretariat a Declaration of Interests (DOI) disclosing potential conflicts of interest that might affect, or might reasonably be perceived to affect, their objectivity and independence in relation to the subject of the meeting. All ACVVR members participated in their individual capacities and not as representatives of their countries, governments or organizations. WHO reviewed each of the DOIs and concluded that none could give rise to a potential or reasonably perceived conflict of interest related to the subjects to be discussed.

The objectives of the meeting were to:

- review progress of approved research with live variola virus;
- discuss new research proposals for 2024; and
- discuss preparation for the seventy-seventh World Health Assembly (2024)

Dr Sylvie Briand, Director, Epidemic and Pandemic Preparedness and Prevention, WHO Health Emergencies Programme welcomed all participants. She commended the committee’s twenty-five years of dedicated work, emphasizing the pivotal role of smallpox countermeasures in enabling many Member States to respond to the mpox outbreak. Key lessons from the mpox outbreak which will inform preparation for the re-emergence of smallpox or another pathogenic orthopoxvirus (OPXV) include:

Community engagement: Continuous engagement with communities before, during, and after crises is crucial. Trust is a cornerstone for managing epidemics and pandemics, which emphasizes the need to build trust proactively rather than wait for the next emergency.

Diagnostic capacity: Having robust diagnostic capabilities is essential and necessary to halt disease transmission and initiate appropriate treatment.

Medical countermeasures: Vaccines play a crucial role in reducing the impact of infectious disease. Continued research for new medical countermeasures, such as diagnostics, vaccines and therapeutics is imperative for better preparedness and response to health threats as is the equitable deployment of them.

She expressed gratitude to all partners for their support and highlighted challenges countries have faced with numerous emergencies including COVID-19 and mpox. She closed by requesting recommendations from the Committee regarding the role of future variola virus research which would be discussed at the Health Assembly in 2024.

Dr David Ulaeto, Committee chair, introduced the meeting agenda. He thanked Committee members for the detailed feedback received on research proposals submitted to WHO by the WHO Collaborating Centres. He reminded members that the focus of the Committee1 was to oversee research on vaccines, antivirals and diagnostics, oversee biosafety inspections of the variola virus repositories, and advise on the timing of

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1 Terms of reference. Advisory Committee on Variola Virus Research. Available at: https://www.who.int/docs/default-source/documents/health-topics/smallpox/tors-acvvr.pdf
destruction of variola virus stocks. He emphasized that without the work of the Committee and stakeholders, only traditional vaccines might have been available when the global mpox outbreak began in 2022. Despite the work of the Committee generating a clear public health benefit, the mpox outbreak still demonstrated gaps and vulnerabilities in the ability to respond to a serious orthopoxvirus event. In the context of the global mpox outbreak and COVID-19 pandemic, he asked that members consider the original objectives set for the Committee and how to achieve them.

1.1 Secretariat report

Dr Rosamund Lewis, Head of the Smallpox Secretariat, WHO Health Emergencies Programme, welcomed members and all participants. New and continuing members were to make recommendations to WHO in line with World Health Assembly (WHA) resolutions WHA51.10, WHA55.16, and WHA60.1 in their personal capacity as experts. Interests declared by Committee members concerned working for their respective government agencies.2

Dr Lewis introduced the report from the WHO Smallpox Secretariat. The research programme set in collaboration with the United States Centers for Disease Control and Prevention (CDC) and the State Research Center of Virology and Biotechnology (VECTOR) and outlined in an updated roadmap for 2020–2023 was reviewed. Ongoing research would be presented as progress reports and new proposals presented for discussion and advice of the Committee.

An update was presented on smallpox and mpox vaccines. On 28 September 2023 the WHO Strategic Advisory Group of Experts (SAGE) on Immunization made recommendations for smallpox vaccination3 in the following areas: the composition of WHO vaccine reserve (physical and pledged), for which inclusion of MVA-BN was now recommended; primary preventive use of smallpox vaccines was recommended for laboratory personnel handling orthopoxviruses and outbreak response team members (per national policy); and use of vaccines during a smallpox outbreak for which vaccination was recommended for contacts of cases and other persons at risk of exposure (including for previously vaccinated individuals); and finally that second and third-generation vaccines were recommended for outbreak response, with first-generation vaccines to be considered when the former are not available.

Dr Lewis then provided an update on the global outbreak of mpox which began in May 2022.4 As of 30 September 2023, there were 91,123 confirmed cases and 157 deaths reported from 115 countries in all six WHO regions.5 Given the progress made during the global response along with the decline in cases, the Public Health Emergency of International Concern (PHEIC) declared by the Director-General on 23 July 2022 under the International Health Regulations (IHR) (2005),6 the PHEIC was lifted on 11 May 2023. In accordance with Articles 16 to 18 and 50 to 53 of the IHR, the Director-General issued Standing Recommendations7 on 21 August 2023 valid for one year, during which States Parties are recommended to do the following:

1. Have national mpox plans integrated into broader health systems. Capacities that have been built in resource-limited settings and among marginalized groups should be sustained.

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2 This interest concerned the following: Alexander Agafonov, Supamit Chunsuttiwatt, Hideki Ebihara, Maryam Kamkar, George Korch, Jean-Vivien Mombouli, Mohamed Moussif, Andreas Nitsche, Nir Paran, Wenjie Tan and David Ulaeto.

3 Meeting of the Strategic Advisory Group of Experts on Immunization, September 2023: conclusions and recommendations. Available at: https://www.who.int/publications/i/item/WER-9847-599-620

4 Disease Outbreak News Monkeypox - United Kingdom of Great Britain and Northern Ireland. Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON383

5 All data are available on the platform for the global mpox outbreak. 2022-24 Mpox (Monkeypox) Outbreak: Global Trends. Available: https://worldhealthorg.shinyapps.io/mpx_global/

6 International Health Regulations (2005) Emergency Committee regarding the multi-country outbreak of monkeypox. Available at: https://www.who.int/groups/monkeypox-ihr-emergency-committee

7 Director-General Standing Recommendations for mpox. Available at: https://www.who.int/teams/ihr/ihr-review-committees/review-committee-standing-recommendations-for-mpox
2. Strengthen and sustain testing and surveillance capacity and ensure that new cases of mpox are notified nationally and to WHO.

3. Protect communities through risk communication and community engagement; continue to build trust and fight stigma and discrimination.

4. Invest in research to better understand mpox disease and transmission patterns, and to develop improved vaccines, diagnostics and treatments.

5. Provide travelers with information to protect themselves and others before, during and after travel and refrain from implementing travel-related border health measures.

6. Deliver optimal clinical care for mpox patients, integrated within HIV and STI programmes, with access to treatments and measures to protect health workers and caregivers.

7. Work towards equitable access to safe, effective and quality-assured vaccines, diagnostics and treatments for mpox.

WHO has also developed a Strategic framework on enhancing control and achieving elimination of human-to-human transmission of mpox, in consultation with numerous internal and external stakeholders, with the following objectives:

1. Achieve control of mpox in every context;
2. Advance mpox research and access to countermeasures; and
3. Minimize zoonotic transmission of mpox.

Emerging concerns are that outbreaks of mpox continue to occur in all regions. Notably, in the Democratic Republic of the Congo (DRC) two clusters of mpox due to clade I monkeypox virus (MPXV) involving sexual transmission have been recorded, the first time that this mode of transmission has been noted for this virus clade. Globally, around 50% of persons with mpox are living with HIV and persons with immune suppression continue to be at greater risk of severe disease and death. WHO continues to support enhancing access to diagnostics, vaccines, and therapeutics and supporting regions and countries to address challenges.

Continuing the Secretariat report Dr Kazunobu Kojima, Technical Officer, Health Emergencies Programme, shared that the biosafety and biosecurity inspection of the WHO variola virus repositories and research centres was conducted at the CDC in May 2022 and at VECTOR in October 2023. Both facilities continue to meet WHO biosafety and biosecurity requirements.

Ms Alexandra Hill, Technical Officer, Health Emergencies Programme, shared an update on the inventory of the WHO smallpox vaccine reserve undertaken in January 2023; 2.8 million doses of mainly first-generation smallpox vaccine remain in the physical reserve. Potency testing of reserve vaccines performed in 2022 at the WHO Collaborating Centre on Smallpox Vaccine, the National Institute for Public Health and the Environment (RIVM) of the Kingdom of the Netherlands had shown that vaccines retain their potency according to WHO standards. In April 2023, the first working group was convened to review and update potency testing protocols for all smallpox vaccines including 3rd generation vaccines.

In 2022, prior to the global mpox outbreak, WHO had initiated procurement of tecovirimat for proof-of-concept work for small scale ad hoc requirements. When the mpox outbreak began, the reserve was made available to all 6 WHO regions for compassionate use for treatment of mpox. This has been accessed by four countries, Brazil, Chile, Sri Lanka, Thailand. Additionally, SIGA Technologies donated 2500 treatment courses of tecovirimat to WHO to be provided under an expanded access protocol (EAP), with to date, treatment courses allocated and deployed to Thailand. The government of Japan donated 25,000 doses of LC16 vaccine to

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9 2022-23 Mpox (monkeypox) outbreak: Global trends. Available at: https://worldhealthorg.shinyapps.io/mpx_global/

10 The EAP is the Protocol for Monitored Emergency Use of Unregistered and Experimental Interventions (MEURI). Supplies were provided to Thailand in July and August 2023 and January 2024, and to Lebanon in November 2023. The term MEURI is used generically while it is understood that tecovirimat has been licensed for mpox in Europe.
Colombia in April 2023. Discussions on donations of MVA-BN vaccines from the government of Germany are on-going. WHO had commissioned an external evaluation to review the processes and activities for enhancing access through allocation and deployment of medical countermeasures (including vaccine, therapeutics and diagnostics) during the mpox outbreak. This would allow WHO to identify and document good practices, lessons learned and challenges encountered, and propose recommendations for improvement for future emergencies.

Dr Lorenzo Subissi, Technical Officer, Health Emergencies Programme, provided an update on diagnostics and laboratory support to countries. WHO had updated interim guidance on laboratory testing for monkeypox virus (MPXV). In July 2023, two Target Product Profiles (TPPs) were published for in vitro diagnosis of mpox, one for detecting MPXV DNA and the other for detecting OPXV viral antigens in remote and community settings. The outbreak had increased demand for mpox diagnostics and FIND is working with Hôpitaux Universitaires de Genève (HUG) to evaluate point-of-care (POC) diagnostics for analytical sensitivity and limit of detection analysis. The diagnostics undergoing evaluation are POC molecular tests, antigen rapid diagnostic tests, and the CueHealth molecular test for mpox. Access to Clade I isolates had been a barrier to diagnostic development and evaluation of these POC tests.

Data was also presented suggesting that the 2007 and 2022 MPXV sequences, from South Sudan and Sudan respectively, cluster with each other and are significantly different from those found in other central African countries. This suggests that there may be silent circulation of MPXV in Sudan/South Sudan or other blind spots, rather than importation from countries with other lineages of clade I MPXV.

Dr Lewis reviewed the WHO workplan for 2024 which, to ensure that public health benefit should continue to accrue from the work of the Committee, included:

- preparing for discussions on smallpox preparedness and variola virus research at the Seventy-seventh WHA in 2024;
- continuing repository inspections at CDC and VECTOR;
- continuing work to leverage developments in smallpox preparedness for countermeasure reserves, composition, potency testing, protocols, guidelines and engagement with Member States, and continuing to strive for mechanisms to achieve equity;
- continuing work on the global mpox response including policy and technical support, strategic framework, country planning guide, diagnostics and laboratory network meeting, and mpox partnership, with a focus on Africa;
- continuing engagement with SAGE to update recommendations on use of smallpox / mpox vaccines; and
- continuing research and development for therapeutics, diagnostics and vaccines and support to other critical areas of research, including viral origins and animal research, field epidemiological investigations and best approaches to clinical case management.

11 Diagnostic testing for the monkeypox virus (MPXV): interim guidance, 9 November 2023. Available at: https://www.who.int/publications/i/item/who-mpx-laboratory-2023-1
12 Cue mpox molecular test. Available at: https://cuehealth.com/products/mpox-monkeypox-molecular-test/
13 Scientific Advisory Group for the Origins of Novel Pathogens. Available at: https://www.who.int/groups/scientific-advisory-group-on-the-origins-of-novel-pathogens-(sago)
1.2 WHO collaborating centre reports

Report on the variola virus collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for variola virus Strains and DNA at FBRI SRC VB (VECTOR), Rospotrebnadzor, Russian Federation

Dr Alexander Agafonov shared an update on the variola virus collection at the State Research Center of Virology and Biotechnology, Rospotrebnadzor (VECTOR). WHO completed inspection of the variola virus repository and research laboratory at VECTOR in October 2023 and found the variola virus research laboratories were in compliance with national and international requirements and with WHO recommendations from successive biosafety and biosecurity inspections. The variola virus collection comprises 120 primarily unique strains maintained in a dedicated repository in freeze-dried or frozen form. There had been no change in the collection since the report in 2022. To date, full genome sequencing has been completed for seventy-eight strains. Future plans include sequencing of the forty-two remaining isolates.

Regarding replenishment of stocks with non-infectious material derived from live variola virus for diagnostic purposes, Dr Sergei Shchelkunov reported that whole genome analysis of 50 variola virus DNA samples had been undertaken in 2023, 46 of which were previously unsequenced strains. The extracted variola virus DNA would be used at the end of 2023 to evaluate the effectiveness of the medical device: Reagent Kit “Vector-MPC-Rrt-Smallpox.” In 2024, it is proposed to establish the full genome sequences of 20 – 25 variola virus strains available at VECTOR and by 2025 to determine the full genome sequences of the remaining isolates.

Dr Agafonov reported that in 2023, the antiviral effectiveness of 24 new chemically synthesized compounds against variola virus was assessed in Vero cell culture. Studies showed that 23 compounds showed activity against variola virus with a selectivity index (SI) > 8. VECTOR proposed the following projects for WHO approval for 2024:

1. replenishment of stocks with non-infectious material, derived from live variola virus, required for diagnostics development.
2. assessment of the neutralizing activity of the sera of mice against live variola virus following their immunization with the fourth-generation OrthopoxVac vaccine (VACΔ6).
3. study of the protective effectiveness of the fourth-generation OrthopoxVac vaccine (VACΔ6) in a mouse model of smallpox.
4. discovery and testing of novel chemical antivirals for smallpox treatment and prevention.
5. use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies.

Progress reports would be presented for all projects approved for 2024.
Dr Christina Hutson shared an update on the variola virus collection at the United States Centers for Disease Control and Prevention (CDC). On prior inspections, the variola virus research laboratories at CDC were found to be in compliance with national and international requirements and with WHO recommendations. To date, CDC had completed initial assembly and phylogenetic analysis of all isolates with extracted DNA. In 2021, two variola virus samples that had undergone cellular passaging were sequenced; from these samples, mutations found were minimal. In 2022, 40 additional variola virus isolates had been transferred from long term storage in the repository freezer to a biosafety level 4 (BSL-4) facility freezer and storage facility. In 2023, sequencing without propagation was attempted for 40 samples: of these 28 were found to be variola virus and 12 non-variola virus. Additionally in 2023, 24 variola virus genomes were submitted to GenBank. Some of these pose challenges due to missing data such as no collection date or country information.

From November 2022 until September 2023, variola virus had been used 437 times: 40 uses for nucleic acid-based diagnostic assays; 383 uses for smallpox small animal model development (processing samples for/from the animal study); 8 uses for next generation vaccine development; 0 uses for antiviral therapeutic development (tecovirimat) 0 uses for ST-357 development, 6 uses to evaluate the efficacy of other potential antiviral compounds (monoclonal antibodies (mAbs)), 0 uses for protein and DNA-based diagnostic development and 0 uses for mRNA vaccine development.

CDC proposed a series of projects for WHO approval for conduct in 2024, to use live variola virus to:

1. maintain and regenerate non-infectious variola virus derived materials for diagnostic development support (approved 2020);
2. develop protein- and DNA-based diagnostic and detection assays specific for variola virus (approved 2019);
3. support less reactogenic vaccine development: continued evaluation of “third” generation vaccines (approved 2019);
4. evaluate mRNA vaccine development (approved September 2023)
5. characterize effectiveness of antiviral therapeutic tecovirimat (additional data, approved 2019);
6. evaluate antivirals (monoclonal biologics) against variola virus (approved 2019 and amendment approved in 2021);
7. determine whether mice are a suitable animal model for human smallpox (Partially approved 2019; approved 2020; amendment approved 2021)
8. characterize effectiveness of novel antiviral therapeutic ST-357 (approved 2019).16

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14 WHO variola virus repository and collaborating centre inspection reports are available at: https://www.who.int/activities/variola-virus-repository-safety-inspections
15 Another 70 or so isolates had been used to completion in various research projects in the past.
16 The proposal to use live variola virus develop the antiviral therapeutic ST-357 was not approved by WHO for 2024 – see section on antiviral therapeutics.
Discussion: reports from the WHO collaborating centres

The Committee had previously agreed that knowledge of the full genome sequences of all variola virus isolates held in the two collaborating centre repositories was important to improve epidemiological understanding of smallpox for *in silico* assessment of sensitivity and specificity of diagnostic assays; for evaluation of modern molecular techniques for variola virus diagnostics; and for identification of variola virus variants that may be resistant to antiviral drugs in order to help pre-select candidate drugs, if drug discovery were to continue.

Discussion as to whether all isolates should be sequenced was revisited during the meeting. Some committee members believed sequencing all isolates to maximize data for future research was important and that in case of an emergency it would be helpful to have all sequences in both repositories to understand what has happened. Others suggested that although scientifically and strategically interesting, sequencing all strains may not be essential for handling the public health consequences of an outbreak. Nonetheless, there was overall a consensus that achieving full coverage of both repositories in terms of full genome sequencing is worthwhile and essential.

The committee re-visited whether sequencing data should be made available publicly immediately or upon request. Some advocated for public access, highlighting the benefits for the development of medical countermeasures, diagnostic advancements, and promoting transparency and therefore trust, and availability of the information in the event of potential future outbreaks. However, concerns exist regarding biosecurity risks, such as potential unauthorized reconstruction of the virus or circumvention of biosecurity checks. CDC follows prior recommendations of the Committee to make all sequences available on public databases while VECTOR proposes to share as yet unpublished sequences through WHO.
1.3 Research reports and proposals

1.3.1 Antivirals

**VECTOR: discovery and testing of novel chemical antivirals for smallpox treatment and prevention**

Dr Artemiy Sergeev shared an update on research on novel chemical antivirals for smallpox treatment (project approved in 2019). The goal of this study had been to test antiviral activity of chemical compounds using surrogate orthopoxviruses to identify the most effective drugs for further efficacy assessment against live variola virus *in vitro*.

In 2023, 180 chemical compounds of different classes were tested in surrogate orthopoxviruses *in vitro*. The most active compounds were those containing fragments of monoterpenes, fenchone, adamantane, isobornylamine, phenylamides, benzylamides, and a bicyclic scaffold.

The antiviral effectiveness of 24 new chemical compounds against the Ind-3a strain of variola virus was evaluated in Vero cell culture with 23 showing activity. Five derivatives of aryl-hydroxyimidazoles (with SI from 120 – 370), four belong to para-aromatic amides based on 1- and 2- adamantanamines (SI: 180 – 15 790), seven are aromatic amides based on bornylamine and phenylhyamine (SI: 50 – 6 120), and seven are N-acyl derivatives of (+) camphor or (-) fenchone hydrazone (SI: 120 – 470). Compound MS-252 showed the highest activity against variola virus *in vitro* with a SI > 15 790. In 2024 it is proposed to use 20 to 25 compounds with a SI of > 100 to test the antiviral activity in live variola virus in Vero cell culture.

Dr. Sergeev also provided an update on the drug NIOCH-14. As reported in 2022, based on data from pre-clinical and clinical studies, NIOCH-14 was licensed by the Russian Federation Ministry of Health on 4 October 2022 for treatment of smallpox, mpox and cowpox for persons aged 18 -50 years (600 mg twice daily for 14 days). The Marketing Authorization No. LP-008597 was issued on 4 October 2022 and was valid until 1 January 2024, with plans to apply for a one-year extension. In February 2023, the manufacturer of NIOCH-14 (Joint Stock Company – Siberian Center of Pharmacology and Biotechnology, Novosibirsk, Russia) introduced into civilian circulation the first limited batch of the antiviral. More information regarding NIOCH-14 is available.17

**CDC: use of live variola virus to characterize effectiveness of antiviral therapeutic tecovirimat**

Dr Todd Smith presented an update to research which had been requested by the US FDA to further assess sensitivity of variola virus strains to tecovirimat and approved in 2019. In 2022, one isolate from a previously identified but untested amino acid variant was tested for tecovirimat sensitivity and was found to be sensitive, such that eight of 12 identified F13 amino acid variants had been tested and were sensitive to tecovirimat. Variola virus isolates with F13 variants which are not available would be tested in stable cell lines expressing the relevant variola virus F13 protein to evaluate sensitivity to tecovirimat during infection with *Vaccinia virus* not expressing the F13 protein (VACVD13L). In 2023, additional genomes from historical variola virus samples were analyzed for F13L variants, and two were identified. Both new variants, E74K and E353K, are proposed to be tested for sensitivity to tecovirimat in 2024.

A comprehensive update was provided regarding the use of tecovirimat in the context of the multi-country mpox outbreak. Under the expanded access protocol in the USA, 7 563 patients with mpox had received tecovirimat treatment. Among the submitted specimens from 435 patients with suspected clinical resistance, virus was isolated from 83 samples, 68 were phenotyped, and 46 were confirmed to have resistance.

Of the 46 patients with confirmed resistant isolates, 39 were from persons living with HIV (PLWH) all of whom were treated with tecovirimat. Among these, 31 had uncontrolled disease resulting in 10 fatalities. Individuals

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17 State Register of Medicinal Products. Available at: https://grls.rosminzdrav.ru/GRLS.aspx?RegNumber=&MnnR=&IF=&TradeNmR=%D0%9D%D0%98%D0%9E%D0%9A-14&OwnerName=&MnFOrg=&MnFOrgCountry=&isfs=0&regtype=1%2c2%2c3%2c4%2c5%2c6%2c7%2c8&pageSize=10&order=Registered&orderType=desc&pageNum=1
with drug-resistant mpox had been experiencing prolonged illness, with an average of 51 days from diagnosis to sample demonstrating drug resistance. Patients had received extended treatment averaging 39 days (ranging from 14 to 167 days).

Analysis was conducted on sequences from 3,247 isolates, confirming 12 mutations previously associated with resistance in other orthopoxviruses and a novel mutation, T289A. In another study in a patient subject to longitudinal sampling, a sensitive isolate was initially identified; after a month of tecovirimat treatment, resistance was detected which increased over time, suggesting that drug resistance arose during treatment. In other cases, resistance mutations were identified from samples from various anatomical sites. There were also instances of probable forward transmission of resistant strains. For instance, a cluster of 11 epidemiologically linked mpox patients, none of whom received tecovirimat, all exhibited the N267 deletion. In this cluster, none of the patients had severe mpox although four had reduced immune function due to HIV. These findings highlight concerns regarding resistance to tecovirimat, estimated to occur at a frequency of 1-4.5% in mpox patients treated. Development of genomic sequence-based tests for clinical use may be needed.

In 2024, proposals for testing with tecovirimat include completing testing for the two additional variola F13 amino acid variants that had been identified, and resuming the generation of cell lines expressing the two amino acid variants that had not been tested.

CDC: use of live variola virus to characterize effectiveness of novel antiviral therapeutic ST-357

Dr Todd Smith presented an update on the research project approved in 2019 to assess the effectiveness of antiviral therapeutic ST-357 (which targets viral mRNA poly-A polymerase encoded by E1L), a possible antiviral discussed in previous meetings. During the first years of the proposal (2019-2020), ST-357 was screened against three isolates of live variola virus and found to be inhibitory, and two ST-357 analogues were screened against a Vaccinia virus Western Reserve strain. ST-357 has synergistic inhibition with tecovirimat and both could be combined to prevent drug-resistance. However, since 2020, challenges with solubility characteristics of ST-357 had led to deferral of this project until additional analogues become available to assess.

The proposal to use live variola virus to characterize effectiveness of novel antiviral therapeutic ST-357 was not approved by WHO and not presented to the Committee as CDC confirmed that there was no prospect that such work would be done in 2024 due to constraints in identifying new analogues with improved solubility characteristics.

CDC: use of live variola virus to determine whether mice are a suitable animal model for smallpox

Ms Audrey Matheny presented an update on this project (for which initial findings had been published).18 To validate the model for testing new smallpox therapeutics, humanized Hu-CD34 and BLT mice were infected with variola virus, showing systemic infection around days 9 to 10 post-infection, which is approximately 3 to 4 days before mortality, allowing for therapeutic testing in these models.

As previously reported, validation of use of tecovirimat in female hu-CD34 mice with treatment initiation on day 0 of virus challenge had 86% survival, which declined to 50% with later treatment initiation. Early treatment was associated with lower virus titres, especially in secondary sites (nose, skin, tongue). All mice in the group without tecovirimat succumbed to infection. Of three animals euthanized on days 5, 7, and 12 post-infection for weight loss and negative for variola virus DNA by PCR, two had virus neutralizing antibodies. Three animals succumbed to variola virus on days 19, 32 and 33 post-infection with extensive secondary bacterial infection, highlighting a risk of secondary infection which had been common in smallpox. Additionally, 7 animals had necrosis of the adrenal gland (as seen in patients with mpox), indicating this model may mimic what happens with other orthopoxviruses during human infections.

18 This project had been partially approved in 2019 and fully approved in 2020, with an extension approved in 2021.
In 2023, a tecovirimat study in male BLT mice was designed to mirror the hu-CD34 study, with the incorporation of an additional uninfected control group. Mortality was observed across all treatment groups starting five to seven days after the cessation of tecovirimat treatment. Statistically significant differences in median survival were noted between the untreated control group and all three treatment groups, with the latter exhibiting twice the duration of survival. However, no significant differences in the concentration of viral DNA had been found in necropsy tissues among the three treatment groups and the untreated infected control group.

Analysis of viral DNA concentrations in organs revealed consistent patterns across all groups. Following the liver, the order of highest to lowest viral DNA concentrations across groups was observed in the lung, spleen, kidney, nose, skin and testes, while the tongue had the lowest concentration of viral DNA.

Continuing investigations aim to detect viable virus in the collected tissues and conduct neutralization assays on serum samples obtained before and after challenge. Thus far, work shows no notable variance in the detection of viable virus among these tissues.

All infected BLT mice succumbed to the disease with delayed disease progression.

Another goal of the project was to identify early biomarkers of smallpox using different assays (Magpix and Nanostring) to allow for judicious use of medical countermeasures and help slow transmission. Preliminary results presented at the 2022 meeting had shown the highest activated genes were for inflammatory markers IL-8 and IFIT2. The gene for CCL7, a marker for monocyte attraction, was the only gene with significant and relevant activation across time. The two most down regulated genes involved in B-cell regulation (reflecting potential antibody production) were MS4A1 and BLNK. The use of Nanostring technology had facilitated the identification of potential candidates for the early detection of smallpox. Importantly, this information might have relevance beyond variola virus, extending to other orthopoxviruses.

For 2024 proposed research includes completion of sample processing and analysis of the BLT efficacy study and continuation of biomarker analysis using samples from previous studies. Despite high mortality observed in mice in these studies, results suggest the model may be useful in future therapeutic testing against variola virus.

**Tecovirimat: licensing and product update**

Dr Dennis Hruby provided further updates on tecovirimat which targets the VP37 protein (F13L gene) of orthopoxviruses and prevents envelopment and release of virions from the cell. Oral tecovirimat was approved for treatment of smallpox in adults and paediatric patients ≥ 13 kg by the US FDA in July 2018 and by Health Canada in November 2021. It was approved for use against smallpox, mpox and cowpox as well as vaccinia complications by the European Medicines Agency (EMA) in January 2022, and by the United Kingdom Medicines and Healthcare products Regulatory Agency (MHRA) in June 2022. The medication is well tolerated and the most frequently reported adverse reactions are headache and nausea. The USA product shelf life of the oral formulation is 7 years. In 2022, continuing development for oral tecovirimat was reported to include: i) pharmacokinetic clinical studies for powder formulations for children < 13 kg; ii) studies of tecovirimat for post-exposure prophylaxis, and iii) the impact of tecovirimat on the efficacy of MVA-BN vaccine use in post-exposure vaccination. Powder for reconstitution continues to be developed.

In May 2022, an intravenous (IV) formulation of tecovirimat with a shelf life of 42 months was approved by the US FDA for treatment of smallpox in adults and paediatric patients weighing ≥ 3 kg who are too sick or unable to take oral tecovirimat capsules. This had been used off-label during the mpox outbreak in 2022 to treat patients with severe disease. In clinical trials to date, no serious adverse events (SAEs) had been reported and the most frequent adverse reactions were infusion site pain and swelling.

Prior to the global mpox outbreak, a clinical study using tecovirimat for treatment of mpox had begun in the Central African Republic in collaboration with the Institut Pasteur de Bangui and Oxford University. During the rise in global demand for tecovirimat in 2022, SIGA donated numerous courses of treatment. In the US the CDC
distributed over 80,000 bottles and 13,000 vials of IV tecovirimat. SIGA has supplied tecovirimat to 27 countries around the world and is supporting seven randomized controlled clinical trials (RCTs), two observational studies, and two expanded access protocols to collect safety and efficacy data and further characterize mpox. Enrolment for the studies continues; discussions regarding pooling of data continue.

Efforts to advance the drug ST-357 include computer modelling to predict and develop analogues with better solubility profiles and other drug characteristics.

**Brincidofovir: licensing and production update**

Mrs Laura Cochrane provided an update on brincidofovir, an antiviral agent with a different mechanism of action than tecovirimat or NIOCH-14. Emergent BioSolutions produces brincidofovir from bulk drug substance supplied by Chimerix. In June 2021, the US FDA approved the short-course therapeutic in tablet and oral suspension formulations for treatment of smallpox in adults and children, including neonates and infants. Brincidofovir can be stored at room temperature with a shelf life of 48 months (tablet) or 30 months (oral suspension); real time stability studies to extend the shelf life of brincidofovir tablets were ongoing. Brincidofovir had been added to the US national stockpile; Canada had acquired it under a special access programme.

The mpox outbreak had led countries to request brincidofovir. The CDC treatment algorithm for severely immune-suppressed patients with mpox included brincidofovir. The therapeutic is available for treatment of mpox in the USA under an investigational new drug (IND) request. There are two ongoing studies i) a pharmacokinetics bioequivalence study and ii) a FDA post marketing commitment for evaluation of brincidofovir in vitro against mutant strains of orthopoxviruses.

**Regulatory status of smallpox and mpox antivirals**

Dr Patrick Harrington from the US FDA Center for Drug Evaluation and Research, Office of New Drugs provided updates on the regulatory status of antivirals for smallpox. Two antivirals, tecovirimat and brincidofovir, had been approved for smallpox treatment based on the “animal rule”. The FDA continued to support and encourage the development of additional medical countermeasures for smallpox, especially those with different mechanisms of action, in case smallpox were to re-emerge.

While tecovirimat is approved for treatment of mpox by the EMA, there was no equivalent FDA approval as the FDA determined the animal rule to not be a viable regulatory pathway to approve drugs for the treatment of mpox. It was considered ethical and feasible to conduct clinical trials to establish safety and efficacy in mpox. Tecovirimat was available in the USA through a RCT or under an EAP. Brincidofovir was available through an emergency use IND application with eligibility criteria.

**Discussion on antiviral research projects**

The Committee highlighted the Seventy-second World Health Assembly agreement to maintain at least two antiviral agents for smallpox as the primary reason for retaining variola virus for research. The main concerns of the Committee were the early signals of tecovirimat resistance observed during the global mpox outbreak. This implied a need for continuing development of antivirals with different mechanisms of action, to prepare for orthopoxvirus-related disease outbreaks.

Members also expressed concerns about the impact of immune suppression, such as may be present in advanced HIV infection or due to other medical conditions or treatments, on individuals with mpox and their ability to recover. The worries were based on observations of resistance to tecovirimat in individuals with advanced immune suppression, reflected in the genetic evolution of virus strains in individual patients. Committee members considered that addressing how the presence of HIV influences the evaluation of therapeutics and vaccines was important. It was suggested that Member States should consider the possible implications of the signals of viral resistance to tecovirimat observed during the global mpox outbreak.

Additional discussion revolved around various aspects of novel antiviral medications, with a significant emphasis on their targets, modes of action, and distinction from current drugs. The members of the Committee were supportive of the data presented by VECTOR, revealing the identification of 23 new chemical compounds...
effective against the Ind-3a strain of variola virus. It was suggested that having someone with expertise in drug chemistry on the Committee could significantly benefit the programme due to the further investigation needed regarding the mechanisms of action.

Discussion focused on the targets of the novel antiviral drugs and the issue of bioavailability, highlighting the need for further exploration and additional information. Members of the Committee discussed the utility of immune deficient mouse models for testing combination therapy, acknowledging their limitations in mimicking functioning immune systems, leading to a consideration of the identification of orthopoxvirus-related biomarkers. Another point of discussion was the assessment of biomarkers in mice and their relevance to humans, highlighting the potential of humanized mice in translational research, especially concerning the identification of orthopoxvirus-related biomarkers. Specific markers related to immune dysregulation and liver function changes were also topics of discussion, although comprehensive insights into these areas remained limited.

The discussion underscored the necessity for additional and varied antiviral strategies, including combination options to give maximal protection in case of a smallpox event and to delay or slow down the emergence of resistance, the importance of comprehending immune responses, challenges in pinpointing pertinent biomarkers, and the need for additional research to address concerns regarding resistance and immune dysregulation. Recommendations related to this subject are available at the conclusion of this report.

1.3.2 Monoclonal antibodies

Evaluate antivirals against smallpox based on monoclonal antibodies

Dr Artemiy Sergeev shared an update on research to evaluate smallpox antivirals based on mAbs, as approved in 2019. This project was to design and select novel mAbs and test their activity in vitro against live variola virus in cell cultures, after first screening and selecting antibodies in vitro using related orthopoxviruses. This strategy would also help reduce handling of live virus for in vivo studies.

Research goals included: i) developing cell line producers of recombinant mAbs specific for orthopoxviruses based on CHO-K1 host cells; ii) obtaining recombinant variants of some immunodominant proteins of orthopoxviruses; iii) assessing specificity of mAbs for selected target proteins; and iv) evaluating the neutralizing properties of mAbs on Vaccinia virus. Thus, in developing mAbs against orthopoxviruses, advanced recombinant mammalian cell lines had been developed to facilitate production of immunodominant proteins. A plasmid had been engineered to synthesize target proteins, B7 and M1 due to their established roles in eliciting protective immunity.

Following on the work previously reported, producers of B7 and M1 proteins had been developed based on the CHO-K1 cell line. Another endeavour involved isolating peripheral blood mononuclear cells (PBMCs) from vaccinated blood donors to enable identification of variants of B lymphocytes. These variants possess B-cell surface receptors to M1 and B7. B cells were isolated from blood samples of vaccinated volunteers. Using single-cell PCR analysis with reverse transcription, an extensive library of VH/VL antibody sequences was created, yielding a diverse set of 20 different nucleotide sequences of antibody variants. Amplification of genetic material obtained from a single cell was complex due to small quantities and instability of messenger RNA. To produce monoclonal antibodies, integration vectors, pVEAL2 and pVL3, were developed containing nucleotide sequences encoding the constant parts of immunoglobulins. Antibodies A31, B23, H72 and M12B9ch that had been previously produced were tested for the presence of virus neutralizing activity against Vaccinia virus in Vero cell culture in vitro. For the remainder of 2023, it was proposed to test the monoclonal antibodies for neutralizing activity against Variola virus Ind-3a and Butler strains with and without complement in a range of test dilutions from 1/10 to 1/1000.

Proposals for 2024 included the following: It was proposed to develop new variants of human monoclonal antibodies to the B7 and M1 proteins of variola virus. Data would be generated on the neutralizing activity of full-length monoclonal antibodies specific to the immunodominant proteins B7 and M1I3 against variola virus in experiments in vitro (in cell culture).
Use of live variola virus to evaluate antivirals (monoclonal biologics) against variola virus (Approved 2019)

Ms Audrey Matheny provided an update on the development of monoclonal antibodies (mAbs) against variola virus and monkeypox virus. Research focused on targeting both intracellular mature virion (IMV) and extracellular envelope virion (EV) forms of the viruses. In previous years, Vanderbilt University switched to FDA-approved mAb production and four mAb mixes were tested against variola and monkeypox viruses, showing effectiveness. In 2022, two individual mAbs and four mixes provided some protection for mice in a vaccinia virus model. In 2023, Mix 2 and Mix 2* demonstrated effective protection against monkeypox virus in a different mouse model in three groups, each receiving Mix 2, Mix 2* or a control antibody respectively.

CDC also tested humanized and chimeric mAbs, finding comparable neutralization levels. Following on from results previously reported, in 2023 CDC completed in vitro testing of the next iteration of BioFactura Inc. anti-IMV and anti-EV mAbs against variola virus. The anti-IMV mAb and a 2023 cocktail showed neutralization comparable to the previous production run, in the presence and absence of complement. The anti-EV mAb, tested in the presence of complement, showed slightly higher neutralization. These results support that production should continue for these mAbs with in vitro neutralization against VARV tests at critical production steps. In summary, mAb products from partner entities continued to show promising neutralization capacity against orthopoxviruses.

Looking ahead to 2024, proposals involve the continued in vitro testing of mAb formulations to assess additional mixes, continue in vitro testing and screening against additional variola virus strains, and evaluate new candidates from Just Evotec following finalization of legal agreements between the company and CDC. Overall, mAbs products show promising neutralization capacity against orthopoxviruses.

Discussion on research projects related to monoclonal antibodies

Several observations and recommendations were made.

The Committee commended the collaborating centres on progress made in development of monoclonal antibodies.

The Committee deliberated on the rationale for creating variola-specific mAbs and considered potential advantages of adopting pan-orthopoxvirus strategies. The discussion related to the VECTOR proposal for testing new monoclonal antibodies targeting M1 and B7 proteins: it was confirmed that assessment of monoclonals against non-variola ‘surrogate’ orthopoxviruses had been undertaken before testing against variola virus. CDC collaborations with several entities aimed also to develop a smallpox-specific mAb following successful testing against monkeypox virus. In addition, the collaboration with Just Evotec focused on a pan-orthopoxvirus mAb.

The use of humanized antibodies in mouse models to assess immunity against variola virus was also discussed. Based on evaluations performed, humanized mouse models showed good performance in evaluating mAbs against the variola virus.

In summary, consensus was reached that given the insights gleaned from the global mpox outbreak and concerning reports on tecovirimat-resistant viral mutations, the investigation into new therapeutics for orthopoxviruses continues to hold significance. It was the consensus view of the Committee that research on mAbs remained crucial, offering potential in a multi-therapeutic approach to treatment of smallpox and other orthopoxviruses. Recommendations on this subject are provided later in this report.
1.3.3 Vaccines

VECTOR: clinical trials of the anti-smallpox vaccine VACΔ6, including the assessment of the neutralizing activity of vaccinated volunteers’ sera and the sera of vaccinated animals to support development of a fourth-generation smallpox vaccine

Dr Artemiy Sergeev shared an update on VACΔ6, a fourth-generation cell culture-based vaccinia-based vaccine (now also known as OrthopoxVac). OrthopoxVac is a fourth-generation minimally replicating vaccinia virus vaccine developed to prevent smallpox in humans. Previous studies had evaluated the vaccine’s protective effectiveness in animal models against vaccinia virus and ectromelia virus. Data from preclinical and clinical studies have also been previously presented. The VACΔ6 vaccine demonstrated lower reactogenicity than the classical (first-generation) vaccine used in the Russian Federation, while retaining immunogenic properties. OrthopoxVac, was licensed in the Russian Federation on 11 November 2022 for immunization of adults aged 18 to 60 years against smallpox, mpox, and other orthopoxvirus infections (marketing authorization valid to 11 November 2027). The shelf-life at 2-8°C of the product was 5 years. Selection of an industrial partner for vaccine production was ongoing.

Dr. Sergeev introduced a new research proposal for OrthopoxVac for 2024. Previous Committee discussions underscored the importance of variola virus neutralization as a correlate of protection in evaluating vaccines that do not elicit typical pox manifestations on the skin. The most reliable method to assess effectiveness of protective immune response from vaccines involved analyzing the neutralizing activity of antibodies in the sera of vaccinated individuals using variola virus stains. These findings were previously reported.

For 2024, it was proposed to assess variola virus neutralizing activity in mouse sera 30 and 90 days after immunization with OrthopoxVac or a first-generation smallpox vaccine to compare the protective efficacy of each. The study would use a mouse model to assess the vaccine in providing protection against variola virus infection and suppression of viral production in the lungs. The objective is to establish a correlation between the variola virus neutralizing activity in mouse sera from both vaccines and their protective efficacy against smallpox in a mouse model.

Looking ahead to 2025, the proposal would be extended to evaluate immunogenicity of OrthopoxVac and assess duration of protection against smallpox by conducting a variola virus neutralization test 180 days post-immunization.

CDC: use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines (Approved 2019) and study of MVA-BN vaccine in health workers in Tshuapa Province, Democratic Republic of the Congo

Dr Todd Smith provided an update on the study of vaccination against mpox of health workers using MVA-BN vaccine in Tshuapa province, in the Democratic Republic of the Congo (DRC). To evaluate vaccine safety and immunogenicity, vaccine had been administered to study participants on days 0 and 28, with follow-up visits and blood draws at set time-points for two years, along with completion of diaries to document exposures to mpox, vaccine side effects and outcomes. The first cohort of 1000 participants received liquid vaccine, the second cohort of 600 participants received a lyophilized formulation. No adverse events were reported. No cases of mpox were identified of follow-up; one participant was diagnosed with mpox 2.5 years after vaccination.

The sera from cohort 1 vaccinees obtained two years post-vaccination had been screened against vaccinia virus using an enzyme-linked immunosorbent assay (ELISA). Participants with presumed prior smallpox vaccination (based on age) had had a more durable antibody response than vaccine-naïve individuals, whose titres peaked on day 42 post-vaccination and declined thereafter. In 2021, virus neutralizing antibody titres were determined
for a sample of cohort 1 vaccinees against both vaccinia and MPXV. Again, participants with prior smallpox vaccination developed higher and more durable virus neutralizing antibody titres. While seroconversion (a two-fold rise in titre from day 0) on day 42 was similar between the two groups, on day 730, half (51%) of older participants had titres still above the threshold for seroconversion compared to 30% of participants without prior vaccination. Virus neutralization by participant sera yielded very similar results for MPXV and vaccinia viruses.

A follow-up vaccine booster study was undertaken in 2022 in the DRC to assess immunogenicity and to determine if the anamnestic response remained. A third dose of MVA-BN was administered to 166 previous study participants in September 2022. This follow-up took place five years after primary vaccination for the cohort that had received the liquid-frozen vaccine, and three years for the cohort that had received the lyophilized formulation. Results were pending at the time of this report.

The most recently approved project was for testing of blood samples collected more than six months post-vaccination from health workers who had received MVA-BN in the DRC clinical study. The study used PRNT to assess antibody titres, compare these with the highest antibody levels after vaccination, and contrast these findings with other vaccination schedules. In 2023, 38 samples from cohort 1 in the DRC study were evaluated, with 19 having had prior vaccination and 19 being vaccine naive. All samples peaked at day 42, with 82% showing virus neutralizing antibody (VNA) positivity. This percentage declined to 76% by day 180 and to 63% by day 730. Previously vaccinated individuals had more durable titres than vaccine-naive persons (84% vs 79% at day 42, 89% vs 63% at day 180, and 84% vs 42% at day 730.

The naïve and pre-vaccinated groups consisted of 16 naïve individuals and 22 previously vaccinated individuals. This configuration revealed durable VNA titres in the pre-vaccinated group when compared to the naïve group (100% vs 56% at day 42, 95% vs 50% at day 180, and 91% vs 25% at day 730).

In conclusion, antibody levels capable of neutralizing variola virus remained robust over time following vaccination. Those with prior vaccination or pre-existing immunity exhibited more persistent antibody levels compared to naïve individuals. Additionally, the ability to neutralize variola virus appears to differ from the neutralization of other related viruses like vaccinia virus and monkeypox virus. These distinctions could be attributed to variations in the testing methods employed or differences in the specific antigens targeted.

In 2024, research is proposed to complete tests on blood samples from individuals who received MVA-BN vaccines in the DRC clinical trial after a booster, and to complete testing on samples from LC16 vaccine recipients in a different study. These measures aim to provide essential insight for the development, commercialization and use of live-attenuated smallpox vaccines in outbreak settings.

**CDC: vaccine immune response and effectiveness following vaccination with MVA-BN in the US population**

Dr Sathesh Panayampalli provided an update on the immunogenicity and effectiveness of MVA-BN (with the commercial name JYNNEOS). For reference, background was provided regarding a study in the Democratic Republic of Congo (DRC) (see previous section). During the 2022-2023 mpox outbreak in the United States, MVA-BN vaccination was offered to persons at risk. A study was conducted in collaboration with the Washington (DC) health department to determine mpox incidence at the time of vaccination and the immune response to the MVA-BN vaccine in individuals without mpox rash. A study published this year primarily focused on test results from samples taken on the day of vaccination. Out of the 215 participants, 156 (72.6%) were male, 172 (80%) were under 50 years of age, and 25 (11.6%) were persons living with HIV. The vaccine was administered subcutaneously followed by an intradermal second dose or both doses were given intradermally.

Participants were assigned to groups for analysis: naïve and previously vaccinated. In this cohort, the percentage of IgM seroconversion in naïve individuals was higher than observed in the Democratic Republic of Congo, at 75% compared to 43%. IgG seroconversion was around 90%. An anamnestic response was observed in previously vaccinated individuals, with earlier and higher IgG levels across all time points compared to the naïve group. Similar to the DRC study, at the six-month mark IgG positivity was about 68% in naïve individuals. The sera of naïve individuals tested by PRNT showed a neutralizing antibody response at day 45. Immune responses following subcutaneous or intradermal administration were not significantly different.
In both studies (Democratic Republic of Congo and Washington (DC)), a strong anamnestic response was observed in previously vaccinated individuals. To investigate if those vaccinated with MVA-BN would show a similar response to a booster dose, a subset of participants from the first DRC study received a booster MVA-BN dose five years after their initial vaccination. Blood samples taken seven and fourteen days following booster vaccination indicated a robust anamnestic response in all individuals, marked by significantly higher IgG levels on both days, compared to day zero, along with a similar increase in PRNT titres. This response was observed even in individuals who had detectable antibodies at day zero.

The global mpox outbreak offered a unique opportunity to gauge the vaccine effectiveness of MVA-BN against mpox due to emergency deployment of the vaccine. Dr Panayampalli discussed findings on effectiveness of MVA-BN vaccine as pre-exposure prophylaxis in two case-control studies. The Epic Cosmos study carried out in 2022 reported on observed vaccine effectiveness of 36% for one dose and 66% for two doses of MVA-BN. The multi-jurisdictional study in 12 US jurisdictions reported vaccine effectiveness of 75% for one dose and 86% for two doses. No difference was detected in vaccine effectiveness for subcutaneous vs intradermal administration.

In summary, the MVA-BN vaccine showed its peak antibody response at day 42 post-second dose. Subcutaneous and intradermal vaccinations triggered similar responses. There was a strong immune response in those previously vaccinated and a decline in antibodies after 6 months in individuals without prior vaccination. The MVA-BN vaccine effectively reduced the risk of mpox, with two-dose regimen offering higher protection than one dose regardless of the administration route.

Further studies will explore the response to a lyophilized formulation and a seven-year post-vaccination booster. The Washington DC study will continue for two years, examining immune responses in vaccinated individuals and those who had mpox infections. Additional research is directed towards evaluating vaccine effectiveness in immune-suppressed individuals and investigating the duration of protection.

**MVA-BN vaccinia vaccine: research, licensing and production update**

Dr Florian Lienert from Bavarian Nordic provided an update on research and licensing of MVA-BN vaccine. They were getting ready to submit a supplemental Biologics License Application (sBLA) to the FDA for the freeze-dried formulation of MVA-BN. The approved shelf life of MVA-BN had been harmonized across all authorized countries (3 years if stored at -20°C, 5 years if stored at -50°C, and 9 years if stored at -80°C). Regulatory submissions for MVA-BN were taking place in multiple countries.

In the USA, MVA-BN was approved for prevention of smallpox and mpox in 2019. The vaccine had already been approved for prevention of smallpox in Canada and in the EU (in 2013) and this indication had been expanded in Canada (in 2020) and during the global mpox outbreak in the EU and United Kingdom (in 2022) to include prevention of smallpox, mpox and related orthopoxvirus infections and disease. MVA-BN had previously been procured by the governments of Canada, the United Kingdom the USA and other undisclosed countries. The global mpox outbreak increased demand for MVA-BN, leading to availability in over 70 countries. Supply contracts were now in place with the Pan American Health Organization (PAHO) Revolving Fund, the EU, and separately with several European governments and other countries around the world. This effort resulted in the delivery of more than 40 million doses between May 2022 and September 2023, with over 1.8 million doses administered worldwide.

The global mpox outbreak served as a catalyst for smallpox preparedness. Bavarian Nordic has devised a method to increase production capacity of MVA-BN by up to 50 times, contingent on the size of the fermenter to be used, through the development of production in a cell line. Demonstrating feasibility, they are now evaluating potential timelines for regulatory approval. This expanded capacity could assist in the transfer of technology for large-scale production during periods of high demand.

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21 Previously, MVA-BN had mainly been procured for national smallpox vaccine reserves; there was no retail business model in place.
In June 2022, WHO provided interim recommendations on the use of ACAM2000, LC16 or MVA-BN for population groups at risk of mpox during an outbreak. These recommendations were endorsed by SAGE in October 2022.\(^{22}\) WHO also called for vaccine studies to be carried during the global mpox emergency.\(^{23,24}\)

Several real-world safety reports on MVA-BN have been published. The largest one, conducted in the USA from May through October 2022, analyzed a period during which roughly 1 million doses of MVA-BN had been administered. Among adults, reports of serious adverse events (SAE) were rare, and none were reported in persons < 18 years of age who had been vaccinated. The most common adverse events (AEs) were nonserious, primarily relating to injection site reactions, aligning with the safety profile observed in pre-licensure studies.

Various studies with a range of study designs had reported vaccine effectiveness of MVA-BN. These studies estimated effectiveness to be between 36% and 89% after one dose and between 66% and 90% after two doses. In addition, research focusing on immune response revealed that a single dose of MVA-BN prompted low levels of monkeypox virus–specific IgG and neutralizing titres. Conversely, MVA-BN induced a robust response in CD4, CD8, and circulating T follicular helper cells. This suggests that protection conferred by MVA-BN could be mediated by T-cells in addition to the antibody response. There are several ongoing studies to further assess MVA-BN. These studies include:

1. **REMAIN**: A prospective observational trial designed to evaluate the effectiveness of MVA-BN in Spain and Latin America;
2. **SEMVac**: A prospective, non-interventional, multi-center cohort study focused on determining the effectiveness and safety of MVA-BN;
3. **SMART**: A multi-site, cluster randomized trial to establish whether MVA-BN can reduce the burden of illness from mpox in household contacts exposed to confirmed cases in countries where the disease has been historically found.
4. A two-staged phase 2 randomized, open-label trial sponsored by the NIH, comparing the immunogenicity and safety of MVA-BN in adolescents aged 12 to 17 years with that of adults aged 18 to 50 years.

**LC16 vaccine: research, licensing and production update**

**Dr Yasuhiko Shinmura** from KM Biologics provided an update on LC16, a third-generation attenuated freeze-dried minimally-replicating vaccinia virus vaccine against smallpox administered via bifurcated needle and licensed in Japan in 1975 based on clinical data obtained in over 50,000 children. It was kept in reserves in Japan for emergency use in case of a smallpox outbreak; in 2013 the WHO recommended the use of LC16 as a medical countermeasure against smallpox outbreak events. In August 2022, during the mpox outbreak, the indication for LC16 was updated to include the prevention of mpox. Pre-clinical pharmacology studies in non-human primates had demonstrated LC16 prevented MPXV infection\(^{25}\), and induced neutralizing antibody response against various orthopoxviruses in humans illustrating similarities in immunity to variola virus and MPXV\(^{26}\). The approved shelf-life at -20 °C was updated from 4 to 10 years. In Japan, clinical trials to assess efficacy and vaccine effectiveness of LC16 against mpox are underway. These include:

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\(^{22}\) Report of the Strategic Advisory Group of Experts (SAGE) on Immunization meeting held on 3-6 October 2022. Available at: https://www.who.int/publications/i/item/who-wer9801-1-18

\(^{23}\) WHO mpox (monkeypox) research - What are the knowledge gaps and priority research questions? Available at: https://www.who.int/news-room/events/detail/2022/06/02/default-calendar/who-monkeypox-research--what-are-the-knowledge-gaps-and-priority-research-questions

\(^{24}\) WHO mpox (monkeypox) research - What study designs can be used to address the remaining knowledge gaps for mpox vaccines. Available at: https://www.who.int/news-room/events/detail/2022/08/02/default-calendar/who-monkeypox-research---what-study-designs-can-be-used-to-address-the-remaining-knowledge-gaps-for-monkeypox-vaccines

\(^{25}\) Smallpox vaccine safety is dependent on T cells and not B cells. Available at: https://pubmed.ncbi.nlm.nih.gov/21450994/

\(^{26}\) Safety and Immunogenicity of LC16m8, an Attenuated Smallpox Vaccine in Vaccinia-Naive Adults. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3218648/
1. A single-arm study (50 participants, > 20 years of age) of immunogenicity and safety of LC16 as mpox vaccination in healthy adults;

2. An open-label, single-arm study (150 participants > 1 year of age) of efficacy and safety of LC16 vaccine as post-exposure prophylaxis for mpox;

3. A randomized controlled trial (5000 participants > 18 years of age, at high risk of mpox) of LC16 to evaluate the prophylactic effect of LC16 on mpox (this study was recruiting).27

Preliminary data from the second study above on safety and efficacy of LC16 as post-exposure prophylaxis was presented. In the study, six individuals (including two living with HIV) who had close contact with an individual with mpox were vaccinated with LC16 within 14 days of exposure. None of the six showed any symptoms of mpox or related complications for 21 days following their close contact. Adverse events observed were associated with inoculation and included rash, fever, lymphadenopathy and reaction at the site of inoculation. Participants fully recovered during the 28-day observation period. The findings provide early data on use of LC16 for post-exposure prophylaxis in individuals in close contact with persons with mpox.

In December 2022, the Ministry of Health, Labour and Welfare (MHLW) donated 25,000 doses of LC16 vaccine to Colombia in response to the government of Colombia’s request. Preparations for a clinical study to examine effectiveness of LC16 vaccine against mpox in risk populations at risk in Colombia was underway.

An update was given on production of LC16, indicating no concerns related to production operations, facilities, or quality of vaccine. The manufacturer is exploring the potential of transitioning the production cell substrate from primary rabbit kidney cells to a suitable cell line, supported by research grants from Japan’s public research funding agency (AMED/SCARDA). This transition aims to enhance the flexibility and future capacity of LC16.

CDC: use of live variola virus to support mRNA vaccine development; new proposal

Dr Nicolle Baird presented an update on a new proposal to characterize the efficacy of mRNA vaccines. During the COVID-19 pandemic, mRNA vaccines had demonstrated safety and rapid scalability to meet global emergency demands. In response to experiences from the global mpox outbreak, Moderna had developed an mRNA-based subunit vaccine designed to target orthopoxviruses, encoding four antigens specific to the monkeypox virus. Preliminary findings from studies conducted in mice against vaccinia virus suggest comparable efficacy of the mRNA vaccine to a comparator MVA vaccine, outperforming it in certain respects. A dose-ranging study in non-human primates (NHPs) had concluded, and Moderna was leading a Phase I clinical trial in adults in the U.K. which began in August 2023. The study would also evaluate humoral and cellular immune responses.

Evaluating whether the immune responses observed in the NHP study and clinical trial could offer cross-protection against variola virus is important. In off-cycle review by the Committee, WHO had approved a proposal to examine immune sera from both studies to evaluate ability to neutralize live variola virus. The NHP dose-ranging vaccination study concluded, and the samples would be sent to CDC. In 2023, no live variola virus work had been completed on this proposal.

Dr Alec Freyn, Senior Scientist in virology at Moderna provided an update on the development of an mRNA-based orthopoxvirus vaccine known as mRNA-1769. This vaccine was initially formulated based on earlier literature reports evaluating proteins suitable for a subunit vaccine. It combines two MV and two EV proteins in a 1:1:1:1 mass ratio of A29L:M1R:B6R:A35R. Study results were presented involving murine immunogenicity, comparing a 1:10th human dose of MVA-BN with mRNA-1769. Improved immunogenicity and reduced morbidity and mortality after lethal vaccinia virus infection had been observed with both mRNA doses.

In collaboration with U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID) a study was conducted in which NHPs received a prime-boost immunization with mRNA-1769 at 150 mg or the human dose of MVA-BN, followed by a challenge with a lethal dose of monkeypox virus Zaire-197 strain. All animals immunized with either mRNA-1769 or MVA-BN survived MPXV infection, unlike five of six controls who

27 A Randomized Controlled Study to Investigate the Effect of LC16 Vaccine on Preventing the Disease of Mpox. https://jRCT1031230137
succumbed to it. The mRNA-1769 group showed significantly lower lesion numbers (54 lesions vs 607 lesions) and milder disease, resolving ten days sooner than the MVA-BN group (8 days vs 18 days). The MVA-BN groups had more severe cases compared to the mRNA-1769 group (four vs none).

Based on this data, further NHP studies are planned to explore dose escalation of the mRNA-1769 vaccine, various challenge models with different orthopoxviruses and different methods to assess immune sera through neutralization.

In August 2023, a Phase 1 clinical trial of mRNA-1769 was initiated in the United Kingdom. The trial involves dosing at 25 mg, 50 mg and 100 mg in a prime-boost design, and interim analysis results were expected in 2024.

**Discussion on research projects related to vaccines**

The Committee initially focused on the CDC proposal to characterize an mRNA-based subunit vaccine designed to target orthopoxviruses. They questioned whether it would be more pertinent to develop an mRNA vaccine encoding variola virus antigens rather than monkeypox virus antigens. Moderna explained the initial choice of MPXV antigens was in response to the global mpox outbreak and the chosen antigens were those highly conserved across orthopoxviruses. Further, CDC would compare immunogenicity across variola virus, MPXV and vaccinia virus antigens.

Comparisons between mRNA vaccine efficacy and MVA-BN were discussed, exploring the potential impact of antigen abundance on effectiveness and differences in innate immune responses. Moderna explained they were focusing on the four antigens selected, suggesting that higher antibody binding to specific antigens supports this theory. They also confirmed that the four mRNAs were co-formulated within the same nanoparticle.

In relation to the cellular immune response, Moderna detailed its strategy to evaluate CD4 and CD8 T cell responses using intracellular cytokine staining, emphasizing the establishment of a GCLP-compliant assay for this purpose. Additionally, they had implemented an Activation Induced Marker (AIM) assay in their labs to provide a comprehensive analysis of the T-cell response. Notably, there is no intention to assess the innate immune response in the clinical study.

They explained the chosen prime-boost interval aligned with what was used for their SARS-CoV-2 vaccine which showed good results. The Committee commented that the four antigens selected as targets were functionally crucial for viral growth, potentially limiting mutation and critical epitopes. Including multiple MV and EV proteins added redundancy, creating a robust barrier to mutational escape. Finally, despite the possibility of escape, the rate of mutation might be lower due to the functional importance of these antigens. Ancient conservation across orthopoxvirus species supports the hypothesis that these antigens are crucial and do not mutate rapidly. Ongoing bioinformatic analysis of genomes aims to reinforce this argument.

The Committee raised concerns about differences in aerosol transmission between smallpox and mpox, emphasizing the significance of considering mucosal immunity. Moderna noted they had measured IgA in serum and had preliminary data indicating reduction of MPXV levels in throat swabs. Further discussion encompassed evaluating immune response durability in the phase one trial, extending beyond a year, with samples collected at six months and one year post-vaccination. There were queries regarding evaluation of the mRNA vaccines for post-exposure prophylaxis. Moderna expressed interest in such future assessments.

The Committee then considered the MVA-BN studies presented. The CDC and Bavarian Nordic clarified that distinct study designs were employed for different vaccine effectiveness studies. The CDC provided insight into breakthrough infections in Chicago in 2023, emphasizing that persons with mpox who had been vaccinated experienced fewer symptoms.

Given CDC data indicating possibly lower vaccine efficacy in immunocompromised individuals, the Committee discussed the potential for boosting which has also been considered within the US Advisory Committee on Immunization Practices. Boosting the immune system with an additional dose has been investigated in the DRC study and data was currently under review. There was additional discussion of the potential benefits of heterologous vaccine schedules with MVA-BN and ACAM2000 vaccines, which had been the original intention of MVA vaccine development. This approach had not been considered in the United States during the mpox outbreak given the population affected, contraindications, and potential side effects with ACAM2000.
Additional discussion focused on the neutralizing antibody response against variola, vaccinia and monkeypox viruses in individuals vaccinated with MVA-BN. They discussed plans for investigating neutralization of extracellular virus with complement and potential differences in responses against these viruses. CDC confirmed they were examining MPXV neutralization, including extracellular virus neutralization with complement. While cellular responses were not usually the primary focus, efforts were underway to explore T-cell response over a longer duration and assess differences in neutralization among the viruses.

Members of the Committee were encouraged by the Bavarian Nordic update on planned new production methods with potential to enhance production capacity. It was noted that the effect on reducing production costs remained uncertain.

The new VECTOR vaccine research proposals prompted discussion about the limitations of the mouse model proposed, in that it reproduced entry and replication of virus in lung cells without accounting for subsequent developments. In discussion aimed to minimize use of live variola virus, the question as to whether variola virus challenge studies would provide more information than neutralization studies was raised. The committee expressed interest in learning whether OrthopoxVac gene deletions were considered in neutralization tests and suggested conducting longevity studies. Other considerations included the study sample size, the use of a first-generation smallpox vaccine as a comparator, albeit one that is in production with modern laboratory methods to eliminate or neutralize potential contaminants, and the choice of surrogate viruses used for comparison.

Finally, the Committee expressed satisfaction regarding the preliminary data shared on post-exposure vaccination with LC16 for mpox and sought information about larger-scale studies planned. It was confirmed that studies would be conducted in Japan and Colombia, providing a broad geographical context for evaluating vaccine efficacy.

1.3.4 Diagnostics

VECTOR: develop advanced methods for rapid point-of-care diagnostics of orthopoxvirus infections (Approved 2020)

Dr Artemiy Sergeev reminded the Committee that the purpose of this project was to create a sensitive, rapid, easy-to-use, inexpensive, and point-of-care dot immunoassay to detect orthopoxviruses. The target of the study is the reagent kit Rapid POX for rapid immunochemical detection of orthopoxviruses that can be used for primary testing of clinical specimens and environmental samples to determine antigens of vaccinia virus, cowpox virus, monkeypox virus, and variola virus.

The purpose of the work was to assess the sensitivity of the Rapid POX reagent kit when using material containing vaccinia virus, monkeypox virus or variola virus. This generic OPXV immunoassay developed by VECTOR had been validated with vaccinia, ectromelia, rabbitpox, cowpox and monkeypox viruses. Varicella zoster, rubella and measles viruses had been used as heterogeneous controls. In experiments previously reported, the assay had been specific and easy to use with a wait time of 36 minutes for up to five specimens at a time. The kit had enabled detection of virus in clinical material such as blood, skin and organs from animals (rabbits, mice) and from pustules at the vaccination site of recent vaccinees.

In 2023, the sensitivity of the reagent kit for vaccinia and monkeypox viruses had been evaluated in side-by-side experiments. Serial dilutions from 1:100 to 1:6400 had been prepared using culture fluid samples containing these viruses. In all repeated tests involving vaccinia virus and monkeypox virus, the Rapid POX kit demonstrated high sensitivity, successfully detecting both viruses at a dilution of 1:1600 (equivalent to $1.5 \times 10^3$ PFU/ml for vaccinia and $1.3 \times 10^3$ PFU/ml for monkeypox virus).

Proposed evaluation of Rapid POX tests during November and December 2023 would use similar dilutions of culture fluid containing variola virus.
CDC: update on DNA-based orthopoxvirus diagnostics and use of live variola virus to develop protein-based (and DNA-based) diagnostic and detection assays specific for variola virus (Approved 2019)

Dr Christina Hutson provided an update on the nucleic acid-based and protein-based diagnostics. The FDA-cleared/CDC developed orthopoxvirus tests, including the non-variola orthopoxvirus (NVO) test FDA approved in 2005, used during the mpox outbreak, and the variola-specific test FDA approved in 2017 are pre-positioned with Laboratory Response Network (LRN) laboratories in the United States. Challenges may arise with new isolates, as had been observed in the first Alaskapox case where conflicting test outcomes had been reported with the NVO test. CDC considers it important to conduct assessment when new non-variola or variola virus sequences become available to ensure sensitivity and specificity are maintained.

During the mpox outbreak, modifications were made to the NVO FDA cleared test to provide options for laboratories. Adaptations included bridging to an additional master mix, a new PCR platform, and an automated DNA extraction platform, essential to cater to the number of cases during the outbreak. In addition, tests had been provided to commercial labs throughout the country.

For 2024 plans involve performing similar bridging studies to those done for the NVO to amend the variola virus FDA cleared test. Additional PCR platforms were being sourced to explore testing options as previous platforms would no longer be supported by manufacturers. Continuing collaboration with LRN laboratories involves providing challenge panels to those laboratories performing orthopoxvirus testing and performing training on the clinical algorithm and enhance readiness in the event of a smallpox outbreak.

Dr Hutson presented an overview of the use of the GeneXpert® platform in the Democratic Republic of the Congo for testing different orthopoxviruses. A collaboration between CDC and BioGx resulted in the design of a multiplex test for use on the GeneXpert® platform that detected monkeypox virus, orthopoxviruses in general, and varicella zoster virus and showed good sensitivity and specificity in laboratory trials. In field tests on suspected mpox cases in Tshuapa and Tshopo provinces since 2022, preliminary findings from 199 samples had shown 84% positive for orthopoxvirus and monkeypox virus, and 7% positive for varicella zoster virus. Confirmatory PCR results on 242 samples at the DRC national reference laboratory (INRB) closely mirrored these findings, with 86% positive for orthopoxvirus and 7% positive for varicella zoster virus. These results indicate the platform’s potential for differentiation between varicella zoster virus and monkeypox virus, demonstrating its practical use with further analysis proposed for 2024.

The presentation highlighted ongoing collaborations, notably with Tetracore. They introduced a new real-time PCR thermocycler (the T-COR), akin to the GeneXpert with an additional benefit but designed for field settings of a four-hour battery life. Collaborating with Tetracore included sending 45 residual samples from the mpox outbreak, resulting in 100% positive concordance. This underscores the potential for exploring these technologies further in smallpox and mpox testing.

For protein-based diagnostics, the update highlighted several collaborative efforts. A continued partnership with Tetracore involved the examination of two CDC-developed antibodies alongside 45 clinical residual samples from the mpox outbreak. The findings showed 75.5% positivity with at least one antibody and 13% positivity with three antibodies (including the two from CDC), indicating room for improvement. Adjustments to enhance the results include modifications to sample preparation to incorporate fresh samples and increase sample volume to achieve better outcomes.

A new study from USAMRIID28 had demonstrated initial success in developing an immunodiagnostic assay for mpox. Their tests using CDC-shared monoclonal antibodies displayed high specificity for MPXV in laboratory isolates and NHP serum. Similarly, a new venture with the University of California San Diego (UCSD) focused on development of an optical biosensor-based detection method known as pixel-diversity Interferometric Reflectance Imaging Sensor (PD-IRIS). This sensor used an antibody chip where particle size determines a

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positive or negative result. Preliminary outcomes of this low-cost, low-tech instrument were encouraging; collaboration aims to optimize this assay for potential future applications.

The update also addressed the lateral flow assay developed by CDC. Despite initial optimism due to promising results in the laboratory, the assay’s deployment in the field revealed a lower sensitivity of 33%, detecting only nine out of 27 mpox cases. Trial of newer, more sensitive technologies could lead to field assessment in future.

Development of the rapid loop-mediated isothermal amplification (LAMP) test in collaboration with Arizona State University had progressed. The focus was now on three targets: Clade II MPXV, pan-monkeypox virus, and pan-orthopoxvirus, each demonstrating the expected specificity on viruses tested thus far. Sensitivity assessments showed over 94% sensitivity across clinical samples (n=45) with Ct values ranging from 20 to 35. The prototype cartridge streamlines testing with results available within 60 minutes for all three tests, showing a slight delay compared to standard PCR instruments. Next steps include additional specificity testing for variola virus at the CDC, to optimize a variola-specific target for the cartridge.

In summary, reliable and rapid diagnostic assays are essential for response. Nucleic acid diagnostics must be validated against new sequences while transitioning to new platforms as technology advances. Given the promising results in the DRC, the GeneXpert multiplex assay would allow for use in surveillance activities. Protein-based diagnostics continued to be explored and while the lateral flow assay initially lacked sensitivity, new collaborations resulting from the outbreak offer hope for improved outcomes. Finally, LAMP diagnostics demonstrate flexibility and potential for field deployment, delivering encouraging results.

Going forward to 2024, two proposals would entail several initiatives. CDC aimed to maintain DNA stocks for sensitivity and specificity testing, collaborating with external partners and responding to a USG agency request for testing of a smallpox diagnostic. They would also continue validating reagents and diagnostic platforms to expand use of regulatory-approved tests. Additionally, they proposed to test new commercially available reagents from BioGX for traditional PCR platforms.

Regarding protein-based diagnostics, CDC would maintain non-infectious material for evaluating new collaborations and consider new monoclonals (e.g. Vanderbilt University) being investigated as antivirals. CDC had also been approached by the government of France to examine a lateral flow assay for variola virus detection. The test had demonstrated efficacy against vaccinia and was intended for use with human samples such as serum and whole blood. Testing was proposed for 2024.

Discussion on research related to diagnostics

The Committee discussed the diagnostic kits being developed by the WHO collaborating centres and considered avenues for future work. Members compared the maturity of the diagnostic area to other medical sectors like vaccines and drugs, emphasizing the importance of establishing target product profiles and examining technology readiness. CDC addressed challenges in engaging commercial entities with their data. During the mpox outbreak, tests expanded from the laboratory response network to five commercial labs to improve access beyond public health labs, significantly increasing testing capacity. However, sustaining commercial interest amid declining cases was an ongoing challenge despite Emergency Use Authorization (EUA) for mpox diagnostics. The Committee confirmed interest in a WHO target product profile (TPP) for smallpox diagnostics, drawing on the initiative for mpox.

Further discussions surrounded the use of live variola virus for diagnostic purposes and the impact of different inactivation methods on antibody testing. CDC highlighted variances in antibody binding to gamma-irradiated versus live virus, underscoring the value of maintaining capacity for validating diagnostics with live variola virus.

The value of continuing efforts towards development of point-of-care diagnostics was emphasized; in the meantime, ability to decentralize nucleic acid amplification tests to provincial or field laboratories, such as expanded use of the GeneXpert system, a backpack-sized system requiring minimal infrastructure and technical expertise, was emphasized. Discussions also emphasized the potential use of the VECTOR field test kit given very encouraging findings on sensitivity and specificity of the lateral flow system with an array of purified viruses and artificial samples from bodily fluids, like blood, saliva, and urine.
Overall, the conversation centered on the operational capacities and sensitivities of diagnostic tools across various samples and live viruses, given the increasing concerns around ongoing monkeypox virus circulation both globally and in Africa, and the continuing importance of readiness for the occurrence of smallpox-like events.

1.4 Variola virus research and lessons from the global mpox outbreak

The Committee discussed progress to date on the mandate to cover antiviral drugs, less-reactogenic vaccines, and robust diagnostics for smallpox. The mandate of the Committee has been followed and has provided significant benefits that are apparent in the response to mpox. However, the response to mpox has also shown critical gaps that must be remedied.

The landscape in which the hazard from smallpox sits has changed since the eradication of the disease in 1980. The advent of large-scale DNA synthesis has made the synthetic construction of poxviruses a reality, which means the hazard from smallpox will never truly go away.

In addition, the prevalence of HIV/AIDS and other immune suppressing conditions alters the way viruses interact with humans at both population and individual levels, including how antiviral drugs (which did not exist in 1980) are used. This is clear from the global mpox outbreak where immune suppression is driving the emergence of tecovirimat resistance during prolonged use of the drug in HIV-AIDS patients.

The advent of mRNA vaccines was not predicted when currently licenced less-reactogenic vaccinia-virus based vaccines began development. The Committee was presented reports of preliminary studies of mRNA vaccines for mpox and this initial data suggests formidable potency – although there is no data yet on durability of protection. The Committee notes that immune efficacy of some vaccines can vary depending on the poxvirus used as a challenge, and without live variola virus the utility of mRNA vaccine approaches for smallpox could not be assessed with certainty.

Over recent years natural infections of humans with monkeypox virus, cowpox virus, Cantagalo virus, Alaskapox virus and Akhmeta virus have occurred. Despite extensive poxvirus genome sequencing, including variola virus, and the extensive similarity between the viruses, we do not understand the molecular mechanisms that make variola virus more deadly for humans. The developments in humanised mouse models suggests these mechanisms may soon be open to analysis using variola virus, which would provide a critical resource for surveillance of mpox and any other poxvirus disease which establishes in humans. As the characterisation of humanized mouse models for smallpox proceeds, analyses of this kind may provide greater public health benefits than was previously foreseen.

The impact of mpox

The Committee also considered the impact of the global mpox outbreak and Public Health Emergency of International Concern declared by WHO from 23 July 2022 to 11 May 2023 on the collective understanding of preparedness for smallpox. The global mpox outbreak provides an indicator for how potential responses to a smallpox outbreak would progress. While human-to-human transmission of mpox in the global outbreak has appeared to be primarily through direct physical and intimate contact, smallpox transmission was historically considered to include direct deposition of infectious particles through the respiratory tract (i.e. via respiratory droplets). The COVID-19 pandemic also involved respiratory transmission, and its progression through communities was far wider than was seen with mpox. Smallpox had far greater morbidity and mortality than either mpox or COVID-19. Several points of relevance were apparent:

- Smallpox is likely to spread more widely and effectively in communities than mpox, with greater virulence;
- Studies of smallpox countermeasures with monkeypox virus and animal models have been integral to licensure of the measures, and these countermeasures have been assumed and shown to be relevant to control of mpox;
- The response to mpox is an indicator of potential progress / outcomes in a smallpox event;
Countermeasures to mpox have been a major public health benefit of research with variola virus – which would not otherwise have occurred;

- Antibody based therapeutics, while showing much promise, would only applicable for widespread use in high, but not low, resource environment;
- Undetected or untreated HIV infection and other immune suppressive conditions are far more prevalent in society now than when smallpox was a major public health problem.

The Committee considered the global mpox outbreak in the context of the above points and noted that the response to mpox has demonstrated that:

- Efforts to fully stop human-to-human transmission in non-endemic regions have not been entirely successful thus far;
- Antiviral resistance arises easily, especially in immune compromised patients; and resistant variants are transmissible between humans;
- Persons vaccinated with licenced less-reactogenic vaccinia vaccines have in few cases experienced breakthrough within months of administration particularly in highly immunized groups; while this is consistent with the vaccine effectiveness data beginning to emerge, it does illustrate that current vaccines alone may not suffice to stop a major another major orthopoxvirus outbreak;
- Production scalability of licenced less-reactogenic vaccines is insufficient for a global poxvirus emergency;
- Point-of-care diagnostics are insufficient for outbreak control in low resource environments;
- Equitable global distribution of countermeasures – which should have been a major benefit from research with variola virus – has not been achieved.

This has serious implications for global preparedness for a smallpox event, which the Committee considered in the context of its mandate to cover smallpox antivirals, vaccines, diagnostics, and global benefits. The Committee concludes that:

- Current licenced antivirals may be rapidly overcome by resistant variants and would not likely be capable of halting transmission of smallpox that has spread beyond an initial focus;
- Immunity from currently licenced less-reactogenic vaccines may not be dependably durable, and combined with challenges in scalability, may not be sufficient for halting transmission of smallpox that has spread beyond an initial focus without other measures;
- Point-of-care diagnostics are not sufficient or available for control of community transmission of smallpox in low resource environments;
- Current preparedness and readiness for smallpox are insufficient for equitable global provision of resources in the event of a smallpox outbreak.
2. Programme of research

The purpose of the research overseen by the Committee, as mandated by the World Health Assembly, is to develop medical countermeasures to enhance global preparedness in the event of the re-emergence of smallpox. Continuing retention of live variola virus by the repositories of the WHO collaborating centres has been temporarily authorized for this purpose insofar as the research and countermeasures developed are of public health benefit for humanity.

The proposed research programme for the period 2020–2023 outlined at the twenty-fourth meeting of the Committee was presented in the report of that meeting. At the present 25th meeting of the Committee, it was agreed to entertain proposals from the collaborating centres for 2024, ahead of the substantive discussion to be held at the 77th World Health Assembly in May 2024.

The Committee received 12 proposals for new or continuing research, 5 from VECTOR and 7 from CDC. The proposals collectively covered:

- Generation of non-infectious variola virus derived materials for diagnostic development support (CDC and VECTOR)
- Post-licensure studies on tecovirimat antiviral drug (CDC)
- Small molecule antiviral drugs (VECTOR)
- Monoclonal antibody antiviral drugs (CDC and VECTOR)
- Continued evaluation of replication-deficient vaccinia virus-based vaccines (CDC and VECTOR)
- mRNA based vaccine development (CDC)
- Protein based detection and diagnosis assays for variola virus (CDC)
- Continued characterization and refinement of humanized mouse models for variola virus infection (CDC)

Committee members considered the proposals prior to the meeting and gave individual appraisal and views on whether approval is warranted. The Committee considered the proposals collectively during the meeting, querying the proposers where necessary. The Committee recommended all proposals as falling within the mandate of antiviral drugs, vaccines and diagnostics for smallpox; having an essential requirement for use of live variola virus; and providing an essential public health benefit. The Committee therefore recommended that all proposals received for research with live variola virus to be undertaken in 2024 be approved by WHO. Following this meeting, upon additional consideration, WHO issued letters of approval to CDC and VECTOR.

Together with the tables in each annual report and Annex 3 appended to this report, Table 1 below provides a summary record of work carried out under WHO supervision and with WHO approval in the four years since 2020. Further minor adjustments are highlighted in italics in Table 1 to reflect discussions at the 25th meeting of the Committee, offering the reader continuing visibility on the work at the time of the next discussion by Member States at the World Health Assembly in 2024.
**Table 1. Programme of research using live variola virus presented by WHO collaborating centres for 2020 to 2024 (with updates)***

<table>
<thead>
<tr>
<th>Area of work</th>
<th>CDC</th>
<th>VECTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genome sequencing</strong></td>
<td>Complete genome sequencing of 40 isolates. Updated to include isolates sampled from the long-term repository vault in 2022. In 2023, 40 samples were sequenced: of these 28 were found to be variola virus and 12 non-variola virus. In 2023, 24 variola virus genomes were submitted to GenBank.</td>
<td>Complete the genome sequencing of 50 of the remaining 88 isolates (2023). In 2023, isolation of 50 variola virus DNA samples and whole genome analysis was undertaken, 46 of which were previously unsequenced strains. In 2024, sequence 20-25 isolates. In 2025, complete remaining sequencing</td>
</tr>
<tr>
<td>Diagnostics</td>
<td>Adapt and optimize multiplex nucleic acid tests for new platforms and field settings. Continue development and optimization of protein-based tests.</td>
<td>Optimize the design of the immunochemistry test kit and its accessories using OPXV, including Variola and Monkeypox viruses.</td>
</tr>
<tr>
<td><strong>Antivirals</strong></td>
<td>Tecovirimat</td>
<td>NIOCH-14 oral formulation Assisting the oral formulation of NIOCH-14. Complete Phase I clinical trials in 2020 (Done). Complete Phase II and III trials for 2021–2022 (Done). Licensure obtained on 4 October 2022.</td>
</tr>
<tr>
<td></td>
<td>Complete testing of tecovirimat in vitro against variola virus strains with F13L gene mutations, in 2020. For variola virus F13 variants no longer available, use surrogate orthopoxviruses with such mutations or create cell lines expressing F13 protein to evaluate tecovirimat for infection with vaccinia virus strain lacking the F13L gene ST-357</td>
<td>New compounds Test 15 compounds found to be highly active against orthopoxviruses against live variola virus: Complete testing in cell culture in 2020; Complete testing in vivo in 2021–2022. Amended: Test 25 compounds for antiviral activity against variola virus; Complete testing in cell culture in 2023.</td>
</tr>
<tr>
<td>Monoclonal antibodies and antibody mixes</td>
<td>Complete screens of individual and mixes of mAbs to neutralize variola virus within optimized IMV and EV assays in 2020; Assist in creating a new universal poxvirus monoclonal mix and evaluate final products in variola virus PRNTs by 2021; Amended to include new work with a commercial entity, to evaluate mAbs and cocktails in vitro against VARV (after VACV screening).</td>
<td>Monoclonal antibodies and antibody mixes Evaluate antivirals against -variola virus based on monoclonal antibodies. Patent secured for chimeric monoclonal antibody.</td>
</tr>
<tr>
<td><strong>Vaccines</strong></td>
<td>MVA-BN and LC16m8 Finalize efficacy testing on long-term titre samples from MVA-BN and/or LC16m8 vaccine trials (as samples are available).</td>
<td>VACΔ6 (OrthopoxVac) Complete Phase I clinical trials (adults 18–40 years) by December 2019 (Done). Undertake Phase II and III clinical trials in 2020–2021 and assess variola virus neutralizing antibody titres from sera of participants (Done). Licensure obtained on 11 November 2022 as OrthopoxVac.</td>
</tr>
<tr>
<td>mRNA vaccine</td>
<td>Use of live Variola virus to support mRNA vaccine development (new for 2023-2024)</td>
<td>Assessment of the neutralizing activity of mouse sera after immunization with the fourth-generation vaccine OrthopoxVac (VACΔ6) against live variola virus Study of the protective effectiveness of the fourth-generation OrthopoxVac (VACΔ6) vaccine in a mouse model of smallpox</td>
</tr>
<tr>
<td>Animal models</td>
<td>Humanized mouse models Complete the remaining in vitro work on the Hu-BLT mouse model. Continue to assess Hu-BLT and Hu-CD34 models using tecovirimat. (done)</td>
<td></td>
</tr>
</tbody>
</table>

* Edits in italics are new from October 2023 compared to the roadmap agreed in 2022. Note: This multi-year research agenda was presented to the WHO Advisory Committee on Variola Virus Research at their 21st, 22nd, 23rd, 24th and 25th meetings, derived from collaborating centre proposals and presentations.
3. Recommendations

In view of the discussions held by the Committee on progress presented at this meeting, as well as the implications of the global experience with two Public Health Emergencies of International Concern since 2020 — the COVID-19 pandemic and in particular the multi-country outbreak of mpox — the recommendations of the Committee at the twenty-fifth meeting are summarized here.

The Committee recommends that:

1. Further research to develop additional small molecule antiviral drugs is essential for control of a smallpox outbreak and should be undertaken. It is furthermore essential that antivirals be validated and licensed for use in combination therapies;
2. Further research on scalable less-reactogenic vaccines, including mRNA vaccines, to improve efficacy and durability, is essential for control of a smallpox outbreak, and should be undertaken;
3. Further research on point-of-care diagnostics suitable for use across all resource levels is essential for control of community transmission of smallpox in the event of an outbreak, and should be undertaken;
4. Robust pre-planning for equitable global provision of resources is essential for re-eradication of smallpox in the event of a smallpox outbreak, and should be undertaken;
5. Genome sequencing of all independent isolates in the two collaborating centres should be completed, to inform analysis of current and future antiviral drug targets;
6. In addition, it is essential to ensure that the investment in variola virus research for smallpox preparedness continues to be fully leveraged to offer solutions for mpox outbreaks in endemic countries.

The Committee discussed the requirements for use of live variola virus that arise from these recommendations and concluded that live variola virus is essential for delivery of recommendations 1, 2, and 3. These recommendations cannot be fulfilled without access to live variola virus. The Committee also notes the timescales for taking antiviral drugs such as tecovirimat from pre-clinical research to licensure. It is the view of the Committee that development and licensure of additional antiviral drugs is not a short-term effort, and live variola virus will be required for years in order to facilitate this. In addition, with respect to vaccines, the Committee notes the strong performance of mRNA-based vaccines in preliminary studies, and also that experience with Covid19 mRNA vaccines demonstrated less than optimal durability of response. The Committee considered there is a clear need for research on assessing and improving the durability of protection conferred by 3rd and subsequent generation vaccines.

Therefore, specific recommendations are as follows.

General

7. Approve project proposals on diagnostics, vaccines, and therapeutics presented by CDC and VECTOR for 2024 (WHO);
8. Undertake robust pre-planning for equitable global provision of resources in the event of a smallpox outbreak (WHO and Member States).

Antivirals

9. Continue research, development and discovery of small molecule antivirals for use by themselves or in combination (WHO and WHO collaborating centres).
Vaccines

10. Continue efforts to characterize the effectiveness against other OPXV of smallpox vaccines approved or in development, and support studies particularly against mpox in field settings (WHO and WHO collaborating centres).

11. Continue research on scalable less-reactogenic vaccines, including mRNA vaccines to improve efficacy and durability which is essential for control of a smallpox outbreak (WHO and WHO collaborating centres).

Diagnostics

12. Continue to work towards development of protein-based OPXV diagnostics with continuing focus on approaches that do not require the use of live variola virus, noting that development of nucleic acid-based diagnostics do not require the use of live variola virus (WHO collaborating centres).

13. Continue to work towards development and validation of (rapid, point-of-care) OPXV diagnostic tests and expedite their availability in a reliable and equitable manner (WHO collaborating centres and WHO).

14. Continue to build on advances in diagnostics for development of point-of-care tests for mpox for use in all resource settings (WHO collaborating centres).

15. Consider development of target product profiles for smallpox diagnostics (WHO).
Annexes

Annex 1 | Agenda

Objectives of the meeting

1. Review progress of approved research with live variola virus;
2. Discuss new research proposals for 2024; and
3. Discuss preparation for WHA77 (2024).

Wednesday 25 October 2023

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 1. Opening and report of the WHO Smallpox Secretariat</th>
</tr>
</thead>
<tbody>
<tr>
<td>12h30</td>
<td>Opening remarks</td>
</tr>
<tr>
<td></td>
<td>Dr S. Briand, WHO Health Emergencies Programme</td>
</tr>
<tr>
<td>12h45</td>
<td>Roll call, Declarations of Interest</td>
</tr>
<tr>
<td></td>
<td>Report of the Smallpox Secretariat</td>
</tr>
<tr>
<td></td>
<td>Dr R. Lewis, WHO Smallpox Secretariat</td>
</tr>
<tr>
<td>13h30</td>
<td>Discussion</td>
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<td></td>
<td>All</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 2. who collaborating centre reports - variola virus and dna collections</th>
</tr>
</thead>
<tbody>
<tr>
<td>14h00</td>
<td>Summary of proposals for research with live variola virus</td>
</tr>
<tr>
<td></td>
<td>Dr D. Ulaeto, Chair ACVVR</td>
</tr>
<tr>
<td>14h15</td>
<td>Report on the variola virus collection at the WHO Collaborating Centre</td>
</tr>
<tr>
<td></td>
<td>for Orthopoxvirus Diagnosis and Repository for variola virus Strains and DNA</td>
</tr>
<tr>
<td></td>
<td>and Replenishment of stocks with non-infectious material,</td>
</tr>
<tr>
<td></td>
<td>derived from live variola virus, required for diagnostics development</td>
</tr>
<tr>
<td></td>
<td>(Approved 2020); continuation proposal</td>
</tr>
<tr>
<td></td>
<td>Dr A. Agafonov, Director General, FBRI SRC VB VECTOR, Rospotrebnadzor, Russian Federation</td>
</tr>
<tr>
<td></td>
<td>Prof. S. Shchelkunov, FBRI SRC VB VECTOR, Rospotrebnadzor, Russian Federation</td>
</tr>
<tr>
<td>14h35</td>
<td>Report on the variola virus collection at the WHO Collaborating Centre for</td>
</tr>
<tr>
<td></td>
<td>Smallpox and Other Poxviruses – Centers for Disease Control, USA</td>
</tr>
<tr>
<td></td>
<td>and Use of live variola virus to maintain and regenerate non-</td>
</tr>
<tr>
<td></td>
<td>infectious variola virus-derived materials for diagnostic</td>
</tr>
<tr>
<td></td>
<td>development support (Approved 2020); continuation proposal</td>
</tr>
<tr>
<td></td>
<td>Dr C. Hutson, Lead, Virus-Host Molecular Interactions Team (VHMI),</td>
</tr>
<tr>
<td></td>
<td>Centers for Disease Control, Atlanta, Georgia, USA</td>
</tr>
<tr>
<td>14h45</td>
<td>Discussion</td>
</tr>
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<td></td>
<td>All</td>
</tr>
<tr>
<td>15h00</td>
<td>Break (15 minutes)</td>
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<table>
<thead>
<tr>
<th>Time</th>
<th>Session 3. ANTIVIRALS - Progress reports</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Time</td>
<td>Session Title</td>
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<td>-------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>15h15</td>
<td>NIOCH-14; research, licensing and production update and Discovery and testing novel chemical antivirals for smallpox treatment and prevention (Approved 2019); continuation proposal</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>15h30</td>
<td>Use of live variola virus to characterize the effectiveness of antiviral therapeutic tecovirimat (additional data, Approved 2019); continuation proposal</td>
</tr>
<tr>
<td></td>
<td>• novel antiviral therapeutic ST-357 (Approved 2019); no new proposal</td>
</tr>
<tr>
<td></td>
<td>• determine whether mice are a suitable animal model for human smallpox (Approved 2021); continuation proposal</td>
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<td></td>
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</tr>
<tr>
<td>15h50</td>
<td>Tecovirimat and ST-357 – licensing &amp; production update</td>
</tr>
<tr>
<td>16h00</td>
<td>Brincidofovir – licensing &amp; production update</td>
</tr>
<tr>
<td>16h10</td>
<td>Regulatory status of smallpox and monkeypox antivirals</td>
</tr>
<tr>
<td>16h15</td>
<td>Discussion on antivirals research projects</td>
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</table>

**Session 4. MONOCLONAL ANTIBODIES - Progress reports**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Speaker(s)</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>16h30</td>
<td>Evaluation of antivirals against smallpox based on monoclonal antibodies. (Approved 2019), continuation proposal</td>
<td>Dr A. Sergeev.</td>
<td>VECTOR</td>
</tr>
<tr>
<td>16h40</td>
<td>Use of live variola virus to evaluate antivirals (monoclonal biologics) against variola virus (Amendment approved 2022); continuation proposal</td>
<td>Ms A. Matheny.</td>
<td>CDC</td>
</tr>
<tr>
<td>16h50</td>
<td>Discussion on monoclonal antibody research projects</td>
<td>All</td>
<td></td>
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</table>

**17h00**  
Close of day one
Thursday 26 October 2023

<table>
<thead>
<tr>
<th>Session 5. VACCINES - Progress reports</th>
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</thead>
<tbody>
<tr>
<td>13h00 Recap of discussions and recommendations of Day 1</td>
</tr>
<tr>
<td>13h20 Update on OrthopoxVac; research, licensing and production update New research proposals related to additional assessment of OrthopoxVac</td>
</tr>
<tr>
<td>13h35 Vaccine immune response and effectiveness following Jynneos vaccination in the US population</td>
</tr>
<tr>
<td>13h45 Use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines (Approved 2019); new work proposed on booster study samples</td>
</tr>
<tr>
<td>14h00 MVA-BN vaccinia vaccine research, licensing and production update</td>
</tr>
<tr>
<td>14h10 LC 16 vaccine research, licensing and production update</td>
</tr>
<tr>
<td>14h15 Use of live variola virus to support mRNA vaccine development; new proposal</td>
</tr>
<tr>
<td>14h30 Discussion on vaccine research updates, proposals and plans</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Session 6. DIAGNOSTICS - Progress reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>14h50 Development of advanced methods for rapid point-of-care diagnostics of orthopoxvirus infections. (Approved 2020); no new proposal</td>
</tr>
<tr>
<td>15h00 Update on DNA-based orthopoxvirus diagnostics and Use of live variola virus to develop protein-based (and DNA-based) diagnostic and detection assays specific for variola virus (Approved 2019); continuation proposal</td>
</tr>
<tr>
<td>15h10 <strong>Discussion</strong> of diagnostic research updates, plans, next steps, including roadmap for development of diagnostics without use of variola virus</td>
</tr>
<tr>
<td>15h30 Break (10 minutes)</td>
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<table>
<thead>
<tr>
<th>Session 7. Closed session for ACVVR members and advisers</th>
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<tbody>
<tr>
<td>15h40 <strong>Discussion</strong> on live variola virus research agenda 2024, recommendations, and pending issues</td>
</tr>
<tr>
<td>16h00 Draft recommendations on the future use of live variola virus for research in preparation for World Health Assembly 77 (2024)</td>
</tr>
</tbody>
</table>

16h20 Close of day two and ACVVR25
Annex 2  |  Participants

Chair
Dr David Ulaeto, Principal Scientist, Department of Biomedical Sciences, Defence Science and Technology Laboratory (DSTL), Salisbury, United Kingdom

Members
Dr Priya Abraham, Director and Scientist, Indian Council for Medical Research (ICMR)-National Institute of Virology, Pune, Maharashtra, India

*Dr Fatima Aziz, Assistant Manager and Safety Officer, Aga Khan University, Karachi, Pakistan

Dr Supamit Chunsuttiwat, Adviser, Department of Disease Control, Ministry of Public Health, Bangkok, Thailand

Dr Clarissa Damaso, Associate Professor, Lab. Biologia Molecular de Virus, Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Ilha do Fundão - Rio de Janeiro, RJ, Brazil

Dr Inger K. Damon, Former Director, Division of High Consequence Pathogens and Pathology (DHCPP), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America (USA) (retired)

Dr Robert Drillien, Former, Research Scientist, Institute of Genetics and Molecular and Cellular Biology (IGBMC), Illkirch, France

Dr Hideki Ebihara, Director, Department of Virology, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan

Professor Andrew Endy, Professor, Department of Bioengineering, Stanford University, Stanford, California, USA

Professor Mariano Esteban, Director, Depto de Biología celular y molecular, Centro Nacional de Biotecnologia (CSIC), Madrid, Spain

Dr Karupiah Gunasegaran, Associate Professor in Biomedicine, School of Medicine, Faculty of Health, University of Tasmania, Hobart, Australia

Dr Maryam Kamkar, Research Biologist, Public Health Agency of Canada, Ottawa, Canada

Dr George W. Korch, Former Director, National Biodefense Analysis and Countermeasures Center; President, Battelle National Biodefense Institute, Frederick, Maryland, USA

Dr Jean-Vivien Mombouli, Former Director General, Laboratoire National de Santé Publique, Brazzaville, Congo

Dr Mohamed Moussif, Chief Medical Officer and National Coordinator, Training program at the Directorate of Epidemiology and Disease Control, Ministry of Health, Morocco

*Dr Andreas Nitsche, Head of Division, Highly Pathogenic Viruses, Centre for Biological Safety, Robert Koch Institute, Berlin, Germany

Dr Nir Paran, Tenured researcher, Israel Institute for Biological Research, Ness Ziona, Israel

*Professor Geoffrey L. Smith, Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

*Professor Wenjie Tan, Chief and Professor of Biotech Center for Viral Disease Emergency, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China
Invited observers (temporary advisors)

Dr Antonio Alcami, Research Professor, Centro de Biologia Molecular Severo Ochoa, Madrid, Spain

Dr Lorna Leal Alexander, Health Threats and Vaccines Strategy, Advisory Function European Medicines Agency, Amsterdam, Netherlands (Kingdom of the)

Dr Delia A Enria (for STAG-IH), Former Director, Instituto Nacional de Enfermedades, Virales Humanas, Buenos Aires, Argentina

Dr Patrick Harrington, Senior Clinical Virology Reviewer, U.S. Food and Drug Administration Center for Drug Evaluation and Research, Silver Spring, MD, USA

Dr Andrea Hulse, Chief, Clinical Review Branch II, Division of Vaccines and Related, Product Applications, Office of Vaccine Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, USA

Mr Vladimir V. Ryabenko, Head, Department of International Relations, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Russian Federation

Dr Tomoya Saito, Director, Center for Emergency Preparedness and Response (CEPR) National Institute of Infectious Diseases, Japan; Representing the SAGE Smallpox and mpox vaccines working group.

Dr Alexander Sergeev, Lead Research Scientist, Department of “Collection of Microorganisms”, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Russian Federation

Dr Larissa Shishkina, Head, Department of Prevention and Treatment of Highly Hazardous Infections, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Russian Federation

Permanent representatives (WHO collaborating centres)

Dr Alexander Agafonov, Director General for Research, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Russian Federation

Dr Christina L. Hutson, Chief of Poxvirus and Rabies Branch, Division of High Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Invited presenters (WHO collaborating centres)

Dr Nicolle Baird-Lead, Virus host molecular interactions team, Poxvirus and Rabies Branch, Division of High Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Ms Audrey Matheny, Microbiologist, Division of High Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Dr Satesh Panayampalli, Lead, Proteomics and Immunology team, Poxvirus and Rabies Branch, Division of High Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Dr Artemiy Sergeev, Deputy Director General, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Koltsovo, Novosibirsk region, Russian Federation

Professor Sergei N. Shchekunov, Head, Department of Genomic Studies, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Koltsovo, Novosibirsk region, Russian Federation
Dr Todd Smith, Microbiologist, Poxvirus and Rabies Branch, Division of High Consequence Pathogens and Pathology National Center for Emerging and Zoonotic, Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Invited presenters (others)
Mrs Laura Cochrane, VP, Global Medical Affairs, Medical Countermeasures, Emergent Biosolutions, Rockville, Maryland, USA
Dr Kristen Cohen, Director of Clinical Biomarkers, Moderna, Cambridge, Massachusetts, USA
Dr Alec Freyn, Senior Scientist, Moderna, Cambridge, Massachusetts, USA
Dr Dennis Hruby, Chief Scientific Officer, SIGA Technologies Inc., Corvallis, Oregon, USA
Dr Florian Lienert, Director, Global Medical Affairs, Bavarian Nordic, Zug, Switzerland
Mr Yasuhiko Shinmura, Manager, Development Department, R&D Division, Kikuchi Research Center, KM Biologics, Kumamoto, Japan

World Health Organization

Regional offices
Dr Manish Kakkar, Technical Officer, High Threat Pathogens. Regional Office for South-East Asia
* Representatives of other regional offices

Headquarters
Dr Sylvie Briand, Director
Ms Grace Che, Programme Officer
Ms Alexandra Hill, Technical Officer
Dr Krutika Kuppalli, Consultant
Dr Rosamund Lewis, Head, Smallpox Secretariat
Dr Jamie Rylance, Medical Officer
Dr Lorenzo Subissi, Technical Officer

(* Unable to attend)
## Annex 3a. Research proposals presented for 2024 by VECTOR, and WHO approval status

<table>
<thead>
<tr>
<th>Proponent and projects</th>
<th>Yes</th>
<th>No</th>
<th>Majority opinion and notes*</th>
<th>Approval date</th>
</tr>
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<tbody>
<tr>
<td>VECTOR Use of live variola virus to:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1. Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development</td>
<td>16</td>
<td>2</td>
<td>Yes</td>
<td>Approval of continuation December 2023</td>
</tr>
<tr>
<td>2. Assessment of the neutralizing activity of mouse sera after immunization with the fourth-generation vaccine OrthopoxVac (VACΔ6) against live variola virus</td>
<td>13</td>
<td>5</td>
<td>Yes</td>
<td>Approval of new proposal December 2023</td>
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<tr>
<td>3. Study of the protective effectiveness of the fourth-generation OrthopoxVac (VACΔ6) vaccine in a mouse model of smallpox</td>
<td>12</td>
<td>6</td>
<td>Yes</td>
<td>Approval of new proposal December 2023</td>
</tr>
<tr>
<td>4. Discovery and testing of novel chemical antivirals for smallpox treatment and prevention</td>
<td>17</td>
<td>1</td>
<td>Yes</td>
<td>Approval of continuation December 2023</td>
</tr>
<tr>
<td>5. Use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies</td>
<td>14</td>
<td>4</td>
<td>Yes</td>
<td>Approval of continuation December 2023</td>
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</table>

* Concerns expressed by members or recommendations conditional on further discussion were reviewed and discussed in plenary at the 25th meeting of the Advisory Committee.
### Annex 3b. Research proposals presented for 2024 by CDC, and WHO approval status

<table>
<thead>
<tr>
<th>Proponent and projects</th>
<th>Yes</th>
<th>No</th>
<th>Majority opinion and notes*</th>
<th>Approval date</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC Use of live variola virus to:</td>
<td></td>
<td></td>
<td>Recommendation of ACVVR members who reviewed proposals</td>
<td>Recommendation of 25th ACVVR</td>
</tr>
<tr>
<td>1. Use of live variola virus to maintain and regenerate non-infectious variola virus derived materials for diagnostic development support</td>
<td>14</td>
<td>2</td>
<td>Yes</td>
<td>Approval of continuation December 2023</td>
</tr>
<tr>
<td>2. Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus</td>
<td>16</td>
<td>0</td>
<td>Yes</td>
<td>Approval of continuation with additional new work December 2023</td>
</tr>
<tr>
<td>3. Use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines</td>
<td>16</td>
<td>0</td>
<td>Yes</td>
<td>Approval of continuation with additional new work December 2023</td>
</tr>
<tr>
<td>4. Use of live variola virus to support mRNA vaccine development</td>
<td>14</td>
<td>2</td>
<td>Yes</td>
<td>Approval of continuation December 2023</td>
</tr>
<tr>
<td>5. Use of live variola virus to characterize effectiveness of antiviral therapeutic tecovirimat</td>
<td>15</td>
<td>1</td>
<td>Yes</td>
<td>Approval of continuation December 2023</td>
</tr>
<tr>
<td>6. Use of live variola virus to evaluate antivirals (monoclonal antibody biologics) against variola virus</td>
<td>15</td>
<td>1</td>
<td>Yes</td>
<td>Approval of continuation with new work December 2023</td>
</tr>
<tr>
<td>7. Use of live variola virus to determine whether mice are a suitable animal model for human smallpox, providing means to evaluate medical countermeasures against authentic agent</td>
<td>15</td>
<td>1</td>
<td>Yes</td>
<td>Approval of continuation December 2023</td>
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<tr>
<td>8. Use of live variola virus to characterize the novel therapeutic ST-357</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Not approved** October 2023</td>
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</tbody>
</table>

* Concerns expressed by members or recommendations conditional on further discussion were reviewed and discussed in plenary at the 25th meeting of the Advisory Committee. ** The proposal to use live variola virus to characterize effectiveness of novel antiviral therapeutic ST-357 was not approved by WHO and not presented to the Committee as CDC confirmed that there was no prospect that such work would be done in 2024 due to constraints in identifying new analogues with improved solubility characteristics.
Annex 4 | Abstracts of presentations

Abstracts from VECTOR

Report on the variola virus collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for variola virus strains and DNA at FBRI SRC VB VECTOR, Rospotrebnadzor

Agafonov A.P.

WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk region, 630559, Russia

Organization of and experimentation with the Russian variola virus (VARV) collection at the WHO Collaborating Centre (WHO CC) for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at FBRI SRC VB VECTOR, Rospotrebnadzor, is in compliance with national and international requirements, and the WHO recommendations. Based on said documents, working instructions (SOP’s) have been developed that govern the implementation of research as well as all other supporting and monitoring activities. For the handling of accidents and emergencies, response plans are in place to contain possible outbreaks or accidents, and first responder teams were established.

Currently, the variola virus (VARV) collection comprises 120 strains, originating from Europe (EURO), Asia (SEARO), Africa (AFRO), South America (AMRO), and Middle East (EMRO) maintained in a dedicated repository in freeze-dried or frozen form.

In 2023, a study was conducted to extract DNA, without virus culture, from 50 samples of VARV strains maintained in the repository at FBRI SRC VB VECTOR, Rospotrebnadzor, and full-genome sequencing of these extracted VARV DNAs was performed. As a result of the above effort, complete nucleotide sequences of the genomes of all the 50 VARV strains were established.

In addition, in 2023, the antiviral effectiveness of 24 new chemically synthesized compounds against VARV was assessed in Vero cell culture. According to the results of the study, 23 compounds showed activity against VARV, with a selectivity index greater than 8.

Work using live variola virus to evaluate antiviral compounds against smallpox based on monoclonal antibodies and testing the developed advanced methods for rapid (point-of-care) diagnosis of orthopoxvirus infections will be carried out in November - December 2023.

The work with live variola virus is scheduled to be continued in 2024 following the WHO approval of the research projects proposed by FBRI SRC VB VECTOR, Rospotrebnadzor, involving the use of live variola virus:

1. Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development.
2. Assessment of the neutralizing activity of the sera of mice against live variola virus following their immunization with the fourth-generation OrthopoxVac vaccine (VACΔ6).
3. Study of the protective effectiveness of the fourth-generation OrthopoxVac vaccine (VACΔ6) in a mouse model of smallpox.
4. Discovery and testing of novel chemical antivirals for smallpox treatment and prevention.
5. Use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies.
Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development


WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk region, 630559, Russian Federation

The efforts to replenish the collection with non-infectious material derived from live variola virus (VARV) are determined by the need to develop sensitive and highly specific diagnostic tools, and they remain essential up to the present.

In 2023, work was carried out to extract DNA from samples of 50 VARV strains: Butler, Indon-6, Aslam, India 378, Botsw-92, Nep-2, Eth-187, Bangl.-2, Bell, Dub-5, Ind-158, Ind-70, Ken-12, Ken-2, Ken-1, AB-urine, B12/C19, Sher Shah, Bangl.-8, Bangl.-9, Botsw-6, Botsw-1, Congo-126, Congo-148, Eth-115, Eth-184, Botsw-139, Botsw-46, Br-20, Br-4, Eth-100, Eth-148, Eth-215, Eth-4, Eth-44, Ind-166, Ind-2, Ind-204, Indon-1, Nep-75/1, Nepal-56, Botsw-102, Br-5, Bur-2, 13/62, Aziz, Fatma, Ind-80, Kuw-29, Nepal-24, maintained in the repository at FBRI SRC VB VECTOR, Rospotrebnadzor, in the form of frozen cultures (homogenates of CAM of embryonated chicken eggs). DNA extraction was performed without additional virus culture.

Full-genome sequencing of the extracted VARV DNA was conducted using Illumina MiSeq equipment. A phylogenetic analysis of published VARV genomes and those of studied VARV strains has been performed.

The extracted DNAs of VARV strains will be used in November – December 2023 to evaluate the effectiveness of the medical device: Reagent Kit “Vector-MPCRrt-Smallpox” (Marketing Authorization No. RZN 2016/3685).
Discovery and testing of novel chemical antivirals for smallpox treatment and prevention


WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk region, 630559, Russian Federation

The efforts to discover new antiviral drugs for the treatment and prevention of smallpox remain essential up to the present.

In 2023, 180 new chemical compounds of different classes were tested in surrogate orthopoxviruses (vaccinia, cowpox, and ectromelia viruses) in vitro. Among them, there were compounds containing fragments of glycyrrhizin, monoterpenes, adamantane, borneol, camphor and fenchone synthesized at N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the Russian Academy of Sciences (NIOCH, SB RAS); adamantane-containing heterocyclic compounds synthesized at Samara State Technical University (SamSTU); and imidazole derivatives containing quinolines or halophenyls synthesized at D.I. Mendeleev University of Chemical Technology of Russia (D.I. Mendeleev RCTU). Among the tested compounds, there were identified compounds whose selectivity indices (SI) against surrogate orthopoxviruses (vaccinia, cowpox, and ectromelia viruses) were about 100 and greater. The most active compounds against surrogate orthopoxviruses were chemical structures containing fragments of monoterpenes, fenchone, adamantane, isobornylamine, phenylamides, benzylamides, and a bicyclic scaffold.

In 2023, the antiviral effectiveness of 24 new chemical compounds against the Ind-3a strain of variola virus (VARV) was assessed in Vero cell culture. As was shown earlier in 2019-2022, these compounds demonstrated the highest activity in vitro in experiments with vaccinia, cowpox and ectromelia viruses. Cidofovir was used as a reference drug. It was shown that 23 chemical compounds tested showed activity against VARV. Of these, 5 compounds (synthesized by D.I. Mendeleev RCTU) with SI from 120 to 370 are derivatives of aryl-hydroxyimidazoles; 4 compounds (synthesized by NIOCH, SB RAS) with SI from 180 to 15790 belong to para-aromatic amides based on 1- and 2-adamantanamines; 7 compounds (NIOCH, SB RAS) with SI from 50 to 6120 are aromatic amides based on bornylamine and phenchylamine; 7 compounds (NIOCH, SB RAS) with SI from 120 to 470 are N-acyl derivatives of (+)-camphor or (-)-fenchone hydrazone. At the same time, the SI of Cidofovir in relation to VARV reached a value of 16.

Therefore, it is imperative to continue this research in order to discover chemical compounds of different classes that would be promising for the development of drugs against human pathogenic orthopoxviruses, including VARV. To test the antiviral activity of chemical compounds using live VARV in Vero cell culture in 2024, it is proposed to use at least 20 to 25 compounds with the selectivity indices of about 100 or greater discovered as a result of screening their activity against surrogate orthopoxviruses in vitro in 2023.
Use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies

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Producers of B7 and M1 proteins have been developed based on the CHO-K1 cell line. To produce biotinylated recombinant proteins, stable polyclonal cell lines CHO-BirA-M1 and CHO-BirA-B7, previously derived on the basis of the CHO-K1 adhesion cell line, were used. Protein production was carried out on a roller unit in DMEM-F12 culture medium, with the addition of 0.2 mM biotin, 10 mM lithium acetate and 5 mM sodium valproate. The culture medium was replaced 3 times, 200 ml of medium every 5 days, then combined and dialyzed against chromatography buffer (0.1% PBS, 20 mM imidazole, and 0.5 M sodium chloride). Chromatography purification of the target protein was performed according to a standard protocol, with the Ni-NTA sorbent.

Using the produced proteins, the B cells were isolated. For this purpose, blood samples from vaccinated volunteers were used. To create a library of VH/VL antibody sequences, single cell PCR with reverse transcription was used. Amplification of genetic material obtained from a single cell is a complex process, which is complicated due to small quantities and instability of messenger RNA. To produce monoclonal antibodies, integration vectors, pVEAL2 and pVL3, were developed containing nucleotide sequences encoding the constant parts of immunoglobulins.

Antibodies A31, B23, H72 and M12B9ch that had produced previously were tested for the presence of virus neutralizing activity against VACV in Vero cell culture in vitro. The developed monoclonal antibodies will be tested for their neutralizing activity against variola virus in November - December 2023.
Assessment of the neutralizing activity of volunteers’ sera and those of animals following vaccination with the OrthopoxVac vaccine and with new vaccine variants, using variola virus to support the development of less reactogenic fourth-generation smallpox vaccines


WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk region, 630559, Russia

A VACΔ6/OrthopoxVac cell-derived vaccine based on the LIVP strain of Vaccinia virus (VACV) with six directionally disrupted virulence genes was developed at FBRI SRC VB VECTOR, Rospotrebnadzor.

The preclinical studies of the fourth-generation VACΔ6 vaccine in various animal models had demonstrated its high specific activity. In 2018, the neutralizing activity of the sera of animals immunized with smallpox vaccines was assessed against VACV and variola virus (VARV). The sera of animals immunized with the VACΔ6 vaccine under study and the sera of animals immunized with the first-generation vaccine as a comparison were shown to have similar neutralizing activity in experiments using both viruses. In addition, it was also noted that the levels of antibodies neutralizing VARV were lower than those for VACV.

In 2019, an open-label controlled clinical study on the safety and tolerability of the live cell-derived vaccinia-based vaccine (VACΔ6) against smallpox and other orthopoxvirus infections, in volunteers aged 18 to 40 years, was completed. In all the studies, the first-generation smallpox LIVP-based vaccine was used as a control, which is characterized with well-known adverse effects associated with first-generation vaccines. In 2020-2021, a double-blind, comparative, randomized, placebo-controlled clinical trial of the immunogenicity, reactogenicity, and safety of the live cell-derived vaccine based on Vaccinia virus (VACΔ6) against smallpox and other orthopoxvirus infections was conducted in volunteers aged 18-60 years.

In 2021, work began to assess the neutralizing activity of the sera of volunteers in relation to VARV. The initial studies have demonstrated the fourth-generation vaccine, as well as the classical first-generation vaccine, to induce the VARV-neutralizing antibodies in clinical trial participants. It should be noted that the sera of volunteers who were vaccinated with OrthopoxVac (VACΔ6) twice at a dose 106PFU had no significant difference in the geometric mean antibody titers from those found in human volunteers immunized with the classical vaccine.

On 11 November 2022, the VACΔ6 vaccine was licensed in the Russian Federation under the name of OrthopoxVac, for prevention of smallpox, monkeypox, and infections due to other orthopoxviruses.
Development of advanced method for rapid (point-of-care) diagnostics of orthopoxvirus infections


WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk region, 630559, Russian Federation

The purpose of the project “Development of advanced methods for rapid (point-of-care) diagnostics of orthopoxvirus infections” is to create a sensitive, rapid, and easy-to-use point-of-care immunochemical test to detect orthopoxviruses. A stand-alone kit for orthopoxvirus detection had been developed, including synthetic carriers with test and control areas and analytical baths filled with ready-to-use working solutions. The kit makes it possible to perform dot immunoassay within 36 minutes at 20 to 40 °C. The evaluation of the effectiveness of detection of mpox virus (strain V79-1-005), vaccinia virus (strain L-IVP), cowpox virus (strain GRI-90), rabbitpox virus (strain Utrecht), and ectromelia virus (strain K-1) had demonstrated the sensitivity of virus detection in unpurified cell culture preparations to be 103-104 PFU/ml. The test does not detect any cross-reactions with heterogeneous viruses (measles, rubella, and varicella) that cause exanthematous diseases. The kit makes it possible to successfully detect viruses in clinical material from infected animals (rabbits and mice) such as blood, skin rashes, animal organs; it also allows detecting vaccinia virus in the post-vaccination scabs in patients. This kit remains stable at 4 to 8 °C for 18 months (observation period). The completeness of the assay, ease of analysis, and the ability to visually record the results make it possible for this test to be used in off-laboratory settings. Equipment for quantity production of the kits has been created.

It is proposed to test the kit using live variola virus in November – December 2023.
Abstracts from CDC

Report on the variola virus collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention (CDC), Atlanta, GA

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The World Health Organization (WHO) Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention in Atlanta, GA continues to maintain one of two consolidated, international collections of Variola virus strains. The majority of these viruses were originally isolated on embryonated eggs and characterized during the final years of the intensification of the smallpox eradication campaign. The primary virus collection is maintained in two separate freezers, one of which is a back-up freezer remaining largely un-accessed. Secure databases addressing WHO recommendations and U.S. Federal Select Agent Program requirements have been constructed and maintained to track usage of Variola virus. Annual reports on the status of these collections are provided to WHO. No new Variola virus seed pools were added to working stocks between 2022 and 2023. Since the 24th ACVVR meeting, WHO-approved research activities utilizing Variola virus from the inventory have focused on: 1) evaluation of monoclonal antivirals (monoclonal biologics); 2) regeneration of non-infectious material for diagnostic support; 3) determining whether humanized mice are a suitable animal model for human smallpox; and 4) and use for next-generation vaccines. In 2023, sample analysis of original isolates removed in 2022 from the repository freezer was conducted. Sequencing and analysis are being finalized for 40 original isolates removed from the vault freezer in 2022. Sequencing without propagation was attempted for 40 samples in 2023 and 28 of these historical isolates were confirmed to be VARV while 12 were non-VARV. Twenty-four VARV genomes have been successfully submitted to GenBank in 2023. Phylogenetic analysis of 200 unpublished draft VARV genomes (including the 28 new sequences) with 40 published references was completed and the 28 sequences grouped with previously sequenced VARV isolates. The laboratory space was in active use from May 25th, 2022 – April 7th, 2023; the laboratory underwent decontamination prior to preventative maintenance during April 2023 and remained “cold” (inactive) through May 22nd, 2023. During June 2023, the U.S. Federal Select Agent Program conducted an inspection and had no findings. In the United States, Variola virus is a select agent and is subject to the select agent regulations (42 CFR part 73).
Use of live variola virus to characterize effectiveness of antiviral therapeutic (tecovirimat)

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External collaborators: Douglas W. Grosenbach, Dennis E. Hruby, Andrew Russo and Candace Lovejoy, from SIGA Technologies, Inc.

The therapeutic agent TPOXX® (tecovirimat) is approved by the United States Food and Drug Administration (FDA) for treatment of smallpox. TPOXX has been tested extensively in vitro and in vivo against multiple orthopoxviruses. It was the frontline treatment in the United States for the 2022 mpox outbreak. The current study was initiated in 2019 as part of a post-marketing requirement/commitment from the FDA to examine amino acid sequence diversity in the target of TPOXX, the F13 protein, and test the antiviral activity of TPOXX against an expanded panel of variola virus (VARV) isolates. Previously 215 VARV genomes were analyzed identifying 10 F13 amino acid variants. In 2023, additional genomes from historical VARV samples were analyzed for F13 amino acid variants, and two variants were identified. One new variant, E74K (variant 11), is available for testing in 2024. The effect of this mutation is unknown but predicted to be sensitive to TPOXX. The second variant, E353K (variant 12), was previously identified in the MPXV 2022 outbreak strain and was shown to remain sensitive to TPOXX. Previously reported results showed all isolates tested (eight VARV isolates from six amino acid variants) were sensitive to TPOXX using a cytopathic effect (CPE) assay with EC50 range of 0.01–0.03 µM and EC90 range of 0.02–0.15 µM. In 2023, no additional VARV isolates were tested using the CPE assay.

The VARV CPE assay was modified to allow TPOXX resistance testing of clinical specimens from mpox cases. As of July 2023, in total, 124 isolates from 68 patients were tested, and 96 of these isolates from 46 patients were found to have a resistant phenotype; most were associated with severely immunocompromised mpox patients who received multiple courses of TPOXX treatment. Isolates with F13 mutations identified by routine surveillance of patients not treated with TPOXX have remained sensitive. While tecovirimat resistance in immunocompromised patients treated may develop, for isolates that we have analyzed/tested, the frequency of resistant viruses remain low (< 1%) compared to the total number of patients treated. These findings inform our understanding of when tecovirimat resistance is likely to occur and highlight the need for additional OPXV therapeutics.

Another antiviral compound (ST-357 or TTP-018) has been identified targeting the viral mRNA poly-A polymerase encoded by E1L, which is a different target than those of approved therapeutics. During the first year of approved study (2019–2020), the parental compound, ST-357, was screened against three isolates of live VARV, and two ST-357 analogues were screened against vaccinia virus strain Western Reserve. All three VARV isolates were sensitive to ST-357 with half-maximal effective concentrations (EC50) of 0.04–0.05 µM. The two ST-357 analogues showed EC50 of 0.14–0.31 µM compared to 0.055 µM for the parental compound against vaccinia virus. Identification of additional ST-357 analogues has been paused since 2020.

Progress under these proposals and plans for 2024 will be reported at the 25th ACVVR meeting in 2023. In the United States, VARV is subject to the Select Agent Regulations (42 C.F.R. part 73).
Use of live variola virus to determine whether humanized mice are a suitable animal model for human smallpox

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Historically, several animal species were tested for susceptibility to variola virus (VARV) infection; to date, non-human primates (NHPs) are the only non-human animals which exhibit disease. Because of limitations with the NHP model and other surrogate orthopoxvirus models, such as short disease incubation periods which do not resemble human smallpox, these systems are suboptimal for evaluating efficacy of therapeutics. We have shown that humanized mice are a more permissive/representative VARV animal model to facilitate testing of therapeutics. We previously published detailed results showing three types of humanized mice (BLT, hu-CD34 and PBMC) were susceptible to VARV infection. We further characterized the VARV hu-CD34 and BLT mouse strains; the PBMC strain was not used due to late disease progression and early development of graft-versus-host disease. The hu-CD34 and BLT mouse models showed that infection is systemic at day 9-10 post infection, which is approximately 3-4 days before mortality, allowing for therapeutic testing in these models.

Two smallpox antiviral compounds have attained licensure in the United States, TPOXX® (tecovirimat) and Tembexa® (brincidofovir). Our goal was to validate the hu-CD34 and BLT models by demonstrating efficacy of TPOXX in these models. We previously completed validation in hu-CD34 mice treated with TPOXX 0, 1, or 2 days post-VARV challenge. Results showed statistically significant protection for all TPOXX treated groups compared to untreated controls. Virus titers were lower when treatment was started earlier especially in secondary sites such as the skin. As expected for this model, no virus neutralizing antibodies were detected in animals that succumbed or survived. We completed the same validation study with the BLT mouse model in 2023, in which all infected mice eventually succumbed to disease; treated groups showed a significant delay in disease progression (euthanasia on day 19-25 post infection) compared to untreated control group (euthanasia on day 8-11). PCR results of necropsy samples (eight tissues and whole blood) showed no significant difference in viral DNA concentration among treated and untreated groups. Based on these results, it has been determined that the hu-CD34 mice may be the better model for future in vivo testing of anti-virals against VARV.

Another goal is to identify early biomarker(s) of smallpox disease. Biomarker molecular assays, which continued in 2023, used both MAGPIX and NanoString® technologies for the hu-CD34 and BLT models. Preliminary results with hu-CD34 tissues showed a large upregulation of T-cell markers in the liver, a large down regulation of B-cell markers in the spleen, and less down regulation of B-cell markers in the lungs and kidney. Results from BLT tissues are being analyzed.

Progress under this proposal and plans for 2024 will be reported at the 25th ACVVR meeting in October 2023. In the United States, VARV is subject to the Select Agent Regulations (42 C.F.R. part 73).
Evaluation of monoclonal biologics against live variola virus

Investigators: Audrey Matheny, Todd Smith, Nicolle Baird, and Christina Hutson
Previous CDC investigators: Ashley Kondas, Victoria Olson

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Additional external collaborators: Iuliia Gilchuk, James Crowe, Jr., (the Vanderbilt Vaccine Center); Darryl Sampey (BioFactura), and Caren Tidwell (Just Evotec Biologics, Inc).

Therapeutic strategies are important for smallpox outbreak response efforts as well as disease treatment. A monoclonal antibody (mAb) cocktail for orthopoxviruses could be beneficial, as it would include at least two mAbs to target both infectious forms of the virus (extracellular enveloped virions (EV) and intracellular mature virions (IMV)), potentially limiting the likelihood of viral resistance.

After testing mAbs developed by Vanderbilt University using plaque reduction neutralization test (PRNT) assays in the presence/absence of 10% complement, four mixes (Mix 2, Mix 2*, Mix 3, and Mix 4) were developed. In 2021, these mixes were tested against VARV and MPXV IMV and EV. All mixes appeared to be effective against IMV and EV forms of both VARV and MPXV with EC50 values between 0.05-1.9 µg/mL. Following successful animal studies evaluating these mixes against vaccinia virus, CDC evaluated the Mix2 and Mix2* against MPXV using the Cast/EiJ mice. Both mixes showed protection from severe weight loss and mortality compared to the irrelevant control mAb group. Mix2 and Mix2* treatment groups had 87.5% survival compared to 0% in the 2D22 control group. The difference in viral replication (measured in radiance) between a mAb control group and Mix2 and Mix2* groups at the site of inoculation was statistically significant by day 7 post infection. CDC will continue to screen mixes against VARV and MPXV with the PRNT as mixes move through production.

Commercial entities have also begun production of mAbs and sent them to CDC for screening against VARV, which we have previously reported to the ACVVR. The agency BARDA awarded an Advanced Research and Development Contract in 2019 to BioFactura for its Smallpox Biodefense Therapeutic. CDC has continued to evaluate the BioFactura mAbs as they are optimized for production, determining EC50 values against both the IMV and EV forms of VARV for humanized variants (current production runs from clonal stable cell banks) for two mAb drug product components (one anti IMV mAb, and one anti EV mAb) and a mAb drug product containing a mixture of both mAbs. The anti-IMV mAb neutralized the IMV form of VARV by PRNT with EC50 concentrations of <0.02 μg/mL, with or without complement. The EC50 concentration without complement was consistent with previous values using earlier productions runs, while the EC50 concentration with complement was 0.0095 μg/mL higher than previously reported values. The anti-EV mAb neutralized the EV form of VARV by PRNT with EC50 concentrations around 0.13 µg/mL. This value was approximately 0.077 µg/mL lower than previously reported values for earlier production runs of that antibody. The mAb drug product effectively neutralized both the IMV and EV forms of VARV with EC50 concentrations of 0.021 µg/mL and 0.23 µg/mL, respectively. It was concluded that larger scale production should continue for both drug components and proposed that in vitro neutralization against VARV should continue at critical production steps. The company has indicated that a Phase 1 Clinical Trial will start in mid-2025 pending achievement of certain go/no-go milestones.

Progress under this proposal and plans for 2024 will be reported at the 25th ACVVR meeting in 2023.
Vaccine immune response and effectiveness following JYNNEOS vaccination in the US population

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CDC’s Poxvirus and Rabies Branch had previously conducted non-inferiority study in healthcare workers with two-doses of JYNNEOS® vaccine in the Democratic Republic of Congo (DRC). This study was important to determine the effectiveness of JYNNEOS vaccine in a mpox endemic area, assessing the immune response in vaccinated individuals up to 2 years following vaccination, through longitudinal sample collections. A subset of the same cohort received booster JYNNEOS vaccination 5 years after the initial study to determine the anamnestic immune response. The results demonstrated a robust anamnestic response in previously vaccinated (just JYNNEOS or JYNNEOS + childhood smallpox vaccination) by day 7 following the booster dose.

During the multinational mpox outbreak in 2022, a large number of individuals, particularly men who have sex with men (MSM), were infected in 108 non-endemic countries, with a total case count of 89,889 and 142 deaths as of September 14th, 2023. The US was one of the major countries affected by the outbreak, with 30,767 cases and 50 deaths reported as the same date. As a pre-exposure prophylaxis, JYNNEOS vaccination was offered to high-risk individuals throughout the US. To determine the immune response to JYNNEOS in the Washington DC (District of Columbia), CDC partnered with DC Health and enrolled vaccinees who received the vaccine via different routes of administration, as part of a longitudinal study. We have determined antibody response to JYNNEOS vaccination uo to 6 months post vaccination. The results were similar to DRC study, more than 94% participants demonstrated sero-conversion both for binding and neutralizing antibodies. At 6 months post vaccination, there was a significant drop in sero-positivity rate among naïve vaccinated individuals.

In total, more than 1.26 million doses of JYNNEOS have been administered in the US as of September 26, 2023. Several studies were initiated to determine the vaccine effectiveness of JYNNEOS for the prevention of mpox. Two such studies are the Epic Cosmos case-control study and the multi-jurisdictional case-control study. The results from these studies comparing the vaccine effectiveness after either 1 or 2 doses, demonstrated effectiveness at reducing risk of mpox disease. Compared to 1 dose, two doses provided highest protection irrespective of the route of vaccine administration. The effectiveness was higher in immunocompetent individuals compared to those with immune suppression.
Use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines

Investigators: Todd Smith, Audrey Matheny, Nhien Wynn, Christine Hughes, Michael Townsend, Subbian S. Panayampalli, Whitni Davidson, Nicolle Baird and Christina Hutson

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Additional external collaborators: Bavarian Nordic

Development of new vaccines has focused on use of attenuated vaccine strains, such as vaccinia virus (VACV) MVA and LC16m8. These “third” generation vaccines, however, were not tested directly for efficacy against smallpox during the eradication campaign since they were developed towards the end or after smallpox eradication. Evaluations of the ability of vaccinee sera to neutralize the mature virus form of variola virus (VARV) represents the best surrogate measure of efficacy for these vaccines. The role of VARV neutralization as a surrogate marker for vaccine efficacy is particularly valuable for evaluation of vaccines like MVA that do not elicit a major cutaneous reaction or “take”, the traditional measure of smallpox vaccine success. Based, in part, on previous results of an optimized VARV plaque-reduction neutralization test (PRNT) that showed no statistically significant difference in the proportion of individuals achieving a four-fold and eight-fold increase in antibody titer post-vaccination between ACAM2000® and JYNNEOS® (MVA-BN), the United States Food and Drug Administration approved JYNNEOS in 2019 for prevention of smallpox and mpox. All raw data of VARV PRNT assays has been archived for future availability and transparency. JYNNEOS was the frontline vaccine administered in the US during the 2022 multinational mpox outbreak.

A clinical study of MVA-BN vaccine in the Democratic Republic of the Congo, where monkeypox virus (MPXV) clade I remains endemic began in 2017. All serum from approximately 1000 participants vaccinated with MVA-BN (liquid formulation, cohort 1 with blood collection on days 0, 14, 28, 42, and at 6 months, 12 months, 18 months and 24 months) have been tested by IgG and IgM ELISA (VACV). Neutralization testing of a subset of participant serum against VACV and MPXV has been completed. Results show similar levels of seroconversion in all assays compared with prior data; those with previous/childhood vaccination (with a live VACV first-generation vaccine) have higher titers that are more durable through the two-year study while naïve individuals peak quickly after the second dose (Day 42) and then see rapid decline in responses.

Cohort 2 of the study received a lyophilized vaccine formulation. A study of a 5-year booster for cohort 1 participants to measure anamnestic response was also completed. Analysis of the serum from boosted participants by IgG and IgM ELISA (VACV) is underway. A subset of participant sera (n=38) from cohort 1 of the study (days 0, 42, 6 months, and 24 months) was tested for VARV neutralization in 2023. Overall, 82%, 76%, and 64% of participants had detectable virus neutralizing antibody at day 42, 6 months, and 24 months post-vaccination, respectively. When participants were divided by VACV neutralization into Day 0 pre-vaccinated and naïve groups, VARV neutralizing antibody was detected in 84%, 89%, and 84% of pre-vaccinated participants at day 42, 6 months, and 24 months, respectively. In the naïve group, 79%, 63%, and 42% of participants had detectable virus neutralizing antibodies at day 42, 6 months, and 24 months, respectively. Understanding the longevity of humoral immunity will allow for efficient use of vaccine, development of effective strategies, and protection of those most at risk. The progress under this proposal and plans for 2024 will be reported at the 25th ACVVR meeting in 2023. In the United States, VARV is subject to the Select Agent Regulations (42 C.F.R part 73).
Use of live variola virus to support mRNA vaccine development

Investigators: Todd Smith, Audrey Matheny, Nhien Wynn, Christine Hughes, Nicolle Baird, and Christina Hutson

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Additional external collaborators: Kristen Cohen and Alec Freyn from Moderna Therapeutics, Cambridge, MA, United States of America.

The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox somehow re-emerges, which includes the development of safe, effective smallpox vaccines. The United States Food and Drug Administration (FDA) has approved for use two smallpox vaccines, ACAM2000® and JYNNEOS® [Modified Vaccinia Ankara (MVA)]. ACAM2000, while effective, has been associated with significant adverse reactions, particularly in immune-compromised populations, limiting the number of individuals capable of being vaccinated. JYNNEOS poses less risk of adverse events and was recently used as the frontline vaccine for mpox during the 2022 outbreak in the United States. However, breakthrough mpox infections have been noted in vaccinated individuals and because it was developed after the eradication of smallpox, real-world data on the effectiveness of the vaccine against smallpox are limited. Additionally, there were production delays during the mpox outbreak, reinforcing the need for additional safe vaccines. To bolster preparedness against potential smallpox re-emergence, it is critical that additional vaccines are developed that are capable of limiting smallpox disease.

Previous studies have demonstrated the potential of an orthopoxvirus subunit vaccine and as demonstrated by the COVID-19 pandemic, mRNA vaccines are safe and production can be rapidly scaled up to meet the demands of a global emergency. Moderna has begun development of an mRNA-based subunit vaccine encoding four (tetravalent) monkeypox virus (MPXV) antigens. This mRNA vaccine was tested for immunogenicity and protective efficacy against vaccina virus (VACV) in a murine model with the mRNA vaccine outperforming the comparator, MVA vaccine, for both immunogenicity and efficacy. Additionally, recent efficacy studies were completed in the NHP MPXV high-dose IV challenge model. Animals immunized with the mRNA vaccine had 90% fewer lesions compared to the MVA vaccine with a shorter course of disease (approximately 10 days shorter).

Moderna is leading additional pre-clinical (animal) and clinical (human) testing that is currently in progress. A dose-ranging study was completed in the NHP MPXV high-dose IV challenge model, and breadth of vaccine-induced immune response against a broad panel of orthopoxvirus species will be evaluated as part of this study. In parallel, a phase I clinical trial has been initiated to evaluate the safety and immunogenicity of the tetravalent MPXV mRNA vaccine in adults. The VACV and MPXV-specific humoral and cellular immune responses induced by a 2-dose regimen will be measured. Determining whether these immune responses can also cross-protect against variola virus (VARV) is critical for continued development of the vaccine.

CDC will use the plaque reduction neutralization test (PRNT) to evaluate the ability of immune sera (from the dose ranging NHP study and the phase I clinical trial) to neutralize live VARV. Antibody neutralization and binding responses to VACV and MPXV will be done in parallel for both the NHP and human clinical sample sets.

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Progress on variola virus materials utilized for diagnostic (DNA and protein) development support

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The ability to validate nucleic acid-based and protein-based diagnostic capacity is critical for early detection and recognition of smallpox should a bioterrorism incident occur. The consequences of either false negatives, or false positives, would lead to public distrust and significantly impact global public health efforts and response efforts. As recently as 2021, new species of Orthopoxvirus (OPXV) are being identified and can confound current DNA diagnostic assays. Additionally, point of care (POC) assays for antibody or antigen detection inadequately validated can result in tests that are inaccurate and insensitive. The need to maintain variola virus (VARV) DNA and VARV antigen stocks at the WHO Collaborating Centre (WHO CC) for Smallpox and other Poxvirus Infections remains important for the future development and validation of diagnostic assays. In the United States, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73).

Assay validation is substantially more robust when validated with extracted genomic DNA, rather than plasmids expressing the target portions of genomic DNA. For sensitivity analyses, use of virus DNA extracted from purified virions allows a calculation of the limit of detection (LoD). Such materials will continue to be used to validate detection assays from WHO member countries. Additionally, greater understanding of the variability within VARV genomes will be instrumental to understand the sensitivity and specificity of nucleic acid-based diagnostic assays. It is important to note that the discovery of novel virus isolates are not rare occurrences. In 2015, a novel OPXV was discovered in Alaska (Alaskapox virus) that was not detected with the Laboratory Response Network (LRN) non-Variola OPXV real-time PCR diagnostic. Additional cases of Alaskapox occurred in 2020 (one case), 2021 (two cases), and 2022 (one case), demonstrating the need to continually ensure diagnostics maintain sensitivity and specificity with new orthopoxvirus detections.

In 2023, 40 samples from the VARV collections were processed for sequencing without propagation. Twenty-eight of these were confirmed to be VARV and in silico analysis of 4 VARV PCR tests was completed; no mismatches were identified so wet lab testing is not necessary. Three additional PCR tests will be interrogated with the new VARV isolates and wet lab testing will occur if needed. Twenty-four VARV genomes have been successfully submitted to GenBank.

During the 2022 mpox outbreak, CDC developed a bioinformatics toolkit to help laboratories perform sequence triage, annotation, and database submission for monkeypox virus (MPXV). The toolkit is not specific to MPXV, and we are currently evaluating it with other large viral genomes within the OPXV genus.

Diagnostic development has continued over the past year, focusing on validation of new reagents and/or equipment to maintain US Food and Drug Administration (FDA) approval. The COVID-19 and MPXV outbreaks have highlighted the importance of having multiple options for master mixes and reagent manufacturing for critical real-time PCR assays. We continue to evaluate different primer/probe chemistries, master mix reagents, and extraction technologies (focusing on platforms most commonly in use within state and local health laboratories) with FDA approved OPXV assays. In 2022, PRB completed necessary bridging studies from the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument to the QuantStudio Dx platform, as well as the Qiagen EZ1 automated platform and data were submitted to the FDA and added to the 510(k) non-variola orthopoxvirus (NVO) test. In 2023, a study plan to perform similar bridging studies (from 7500 to QSDx) and to add the EZ1 automated extraction platform to the variola 510k were drafted. As part of this study plan, PRB will determine whether the QSDx and EZ1 instruments generate non-inferior results to the currently approved PCR platforms and extraction method, by performing a validation study that will include the following: determining the LoD of the variola 510K assay, demonstrating reproducibility of the variola virus real-time PCR Assay on both the 7500 and QSDx instruments, determining specimen stability for approved sample types.
with the variola virus real-time PCR assay, and performing an extraction platform comparison to compare the EZ1 extraction platform to the currently approved manual extraction method. In addition to the QSDx, it is critical that there are additional PCR platform options available that can be adapted for use with these assays if currently approved PCR platforms are phased out by the manufacturer. PRB has sourced and purchased two newer model real-time PCR instruments to assess their performance and suitability for use with the currently approved OPXV assays. These studies are proposed for 2024. In 2023 CDC worked with the LRN laboratories that perform orthopoxvirus and VARV testing to participate in testing of challenge panels distributed by CDC to ensure the laboratories are ready in the event of a smallpox outbreak. The mock specimens within the challenge panels are marked as low, medium and high-risk for smallpox and the laboratories must test according to risk. Over 80 laboratories participated in the exercise and CDC will be reviewing the results once completed.

We have also developed and improved wet lab and bioinformatic protocols for sequencing OPXV directly from clinical samples using the Oxford Nanopore MinION for rapid results and the Illumina NovaSeq platform for high throughput sequencing of hundreds of samples per week. Using the MinION, we have been able to determine OPXV species and MPXV clade within the first 40 minutes of sequencing. For samples with Ct >30, we have had success with custom and commercial probe-based enrichment approaches for MPXV. We have also scaled up our sequence annotation and submission throughput, have published an annotation and submission pipeline developed for MPXV, and are working to adapt it to VARV and other OPXV. We have made progress resolving tandem repeats and insertion deletion mutations that can be missed using standard assembly and amplicon sequencing approaches.

In late 2020, we optimized the GeneXpert conditions for a multiplex assay to differentiate OPXV and VARV and confirmed an LOD of ~3000 pfu using a mock clinical sample (VARV spiked on a swab). This year we obtained commercially available reagents from the company to test these updated reagents that include MPXV and OPXV signatures. This testing, planned for 2024, will ensure both targets perform well after manufacturer changes were made to the reagents, including ensuring that testing with VARV is still detected with the OPXV generic target and is not detected by the MPXV target. Our work with this instrument in Africa has recently resulted in GeneXpert for OPXV, MPXV, and varicella zoster virus (VZV) now being operational in 2 provinces in the Democratic Republic of the Congo (DRC) with independent validation being performed at Institut National pour la Recherche Biomedicale (INRB). Preliminary results (as of June 2023) for 247 suspected mpox cases were tested with the GeneXpert, and of those cases 199 have results available with 84% being positive for MPXV and OPXV, 2% positive for MPXV and inconclusive for OPXV, 7% positive for VZV and 8% were negative for MPXV, OPXV and VZV. For 242 specimens with confirmatory PCR test results available, there is good concordance with the GeneXpert test with 86% positive for OPXV, 7% positive for VZV and 7% negative for OPXV and VZV. Additional GeneXpert data from 52 cases has been requested to verify OPXV, MPXV, and VZV results. Cases were flagged for review if (1) the reaction failed, (2) any result was inconclusive, (3) GeneXpert OPXV and MPXV results did not match, or (4) GeneXpert and PCR results were discordant. The current project validation period will end in 2024.

We have continued evaluating the use of a lateral flow assay (LFA), Orthopoxvirus BioThreat Alert kit (from Tetracore) for field use in the DRC, where MPXV infections are endemic. An IRB approved OPXV-generic POC test is being used in the DRC to detect MPXV in patient lesion samples. As presented previously, the pilot study completed enrollment of 36 participants in July 2021 with 94% of these patients having their results. Initial results found high specificity but low sensitivity in samples also tested by real-time PCR. There was a reduction in time from rash onset to test result from an average of over 30.3 days to 4.5 days. We tested the utility of this LFA kit using the samples collected during the current mpox outbreak. The results are similar to that observed in DRC, high specificity but low sensitivity. Efforts to analyze missing specimens and publish results are ongoing.

CDC provided the mAbs reactive against MPXV (one of these mAb is highly specific for MPXV-encoded A29 protein) for antigen detection assay development to government, academic and private labs during this mpox outbreak. Preliminary results are promising and CDC will continue these collaborations. The development of a VARV specific antigen detection assay has not proceeded due to the CDC’s mpox engagement and change in priority for the collaborator lab.
We also have continued a collaboration with Arizona State University (ASU) to assess a Loop Mediated Isothermal Amplification (LAMP) assay as a potential point of care test for smallpox. We analyzed OPXV genome sequences and developed LAMP based OPXV generic assays, MPXV generic assay and MPXV clade II specific assays. Those are being validated in the laboratory using the portable system developed at ASU, including testing of residual clinical specimen remainders from mpox cases with a range of Ct values with real-time PCR. We tabulated assay sensitivity for Clade II-specific, pan-MPXV, and pan-OPXV assays benchmarked to 45 unpurified swab eluate samples collected during the USA2022-2023 outbreak.

Sensitivity was calculated as the mean of detected positives divided by total positives for 3 replicates of each sample. Benchmark Ct values are approximate and were provided with the anonymized samples. Clade II-specific target had a calculated sensitivity of 94 ± 4%, sensitivity of the pan-MPXV was 98 ± 0% and for the pan-OPXV sensitivity was 93 ± 4%. CDC also shared the design of the LAMP prototype cartridge (i.e. chip) designed by ASU collaborators which is a 4 channel microfluidic assay cartridge with integrated sample purification using a dye based detection. The time-to-detection heat map for 8 MPXV2022 clinical samples purified and amplified completely on chip, with 3 negative control reactions composed of pooled negative saliva samples was presented. All samples amplified for 90 minutes and the relative speed performance between lab-based and on-chip amplification times for each LAMP assay showing a fairly consistent delay of ~10 minutes for the three LAMP targets compared to the real-time PCR assay. Next steps will include CDC transfer of negative clinical specimens to ASU, transfer of LAMP cartridge to CDC for specificity testing which will include testing of various OPXVs including VARV, optimization of the cartridge prototype by ASU and potentially , creation of a VARV specific target.

Progress under these two proposals (protein-based and regeneration of noninfectious VARV material for diagnostic development) and plans for 2024 will be reported at the 25th ACVVR meeting in 2023.
Abstracts from invited speakers

2023 Update for Brincidofovir (Tembexa®) oral tablets/oral suspension

Laura Cochrane
Emergent Biosolutions

Brincidofovir (BCV) was approved by the United States Food and Drug Administration (FDA) on June 4, 2021, for treatment of smallpox in adults and children, including neonates. The mechanism of action and resistance profile of BCV differs from the approved treatment for smallpox (tecovirimat), allowing it to retain activity against resistant strains. In addition, BCV adds another smallpox antiviral to the US Strategic National Stockpile (SNS), thus satisfying the recommendations from the US Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) to have at least two smallpox antivirals with different mechanisms of action available in the event of a smallpox outbreak. There is an ongoing FDA post-marketing commitment which includes the evaluation of BCV in vitro against mutant strains of orthopoxviruses, and Emergent is also assessing a pharmacokinetic bioequivalence study.

As of October 26, 2022 the United States Strategic National Stockpile now holds BCV. Clinicians with mpox patients necessitating BCV treatment must submit an e-IND request to the US FDA by email.1

Bioequivalence studies: The goal is to evaluate whether Form H and Form II, 100mg BCV tablets are bioequivalent, when given under fasting conditions in healthy adults. Participants will be randomized to each receive one tablet of Form H and one tablet of Form II, 14 days apart and undergo pharmacokinetic testing pre-dose and post-dose to evaluate safety. This is an open-label, single-dose, randomized, two-period, crossover study. A second objective is characterization of plasma BCV pharmacokinetics following single doses of BCV when administered to healthy adult participants. Safety objectives include evaluating the safety of BCV following administration of single dose of 100 mg BCV Form H and BCV Form II tablet in healthy adult participants.2

BCV is made available from the US SNS for treatment of mpox to clinicians who request and obtain an FDA authorized single-patient emergency use IND (e-IND). BCV can be considered for use under an e-IND for treatment of human mpox disease in adults and pediatric patients (including neonates) with positive results of human mpox viral testing who: have severe disease OR are at high risk for progression to severe disease, AND meet either of the following: Experience clinically significant disease progression while receiving tecovirimat or who develop recrudescence (initial improvement followed by worsening) of disease after an initial period of improvement on tecovirimat, OR are otherwise ineligible or have a contraindication for oral or intravenous tecovirimat.3

Emergent remains committed to support clinical orthopoxvirus studies as appropriate to better understand the risk/benefit profile across our broader smallpox portfolio. Emergent is interested in clinical studies with immunocompromised patients.

References:
**Tecovirimat update**

*Dennis E. Hruby, SIGA Technologies, Inc.*

Tecovirimat is an antiviral with efficacy against pathogenic orthopoxviruses, including variola virus. Oral tecovirimat has received regulatory approval from the US FDA, the EMA and the MHRA with additional approvals underway. It has been stockpiled by the US and now a number of other counties around the world. IV tecovirimat has received US FDA approval and is envisioned for use in severely ill patients as a bridge to oral tecovirimat therapy. For pediatric patients under 13 Kg in weight, a powder for reconstitution (PfR) is in the final stages of development.

During the recent mpox global outbreak, tecovirimat, both oral and IV, has been used to treat infected patients. SIGA is participating in a large number clinical trials around the globe, both observational and RCT’s, to demonstrate the safety and effectiveness of tecovirimat in mpox patients. The status of these ongoing activities will be summarized.

**Update on the “third” generation LC16 vaccine**

*Yasuhiko Shinmura, KM Biologics Co., Ltd.*

LC16m8 is an attenuated replication-competent vaccinia virus developed from the Lister strain by serial passaging in primary rabbit kidney cells in the 1970s. LC16m8 strain has low virulence and shows good protective efficacy in animal models. The LC16 vaccine was licensed in Japan in 1975 based on clinical data obtained in ~50,000 children. In 2013 the World Health Organization (WHO) stated that the third generation LC16 vaccine should be recommended for use as a very useful and beneficial medical countermeasure against smallpox outbreak events.

In August 2022, the indication of LC16 vaccine was updated to “Prevention of smallpox and mpox (monkeypox)” and the approved shelf-life at -20°C storage was updated from 4 years to 10 years based on evidence data.

Globally, the LC16 vaccine is the sole smallpox and monkeypox vaccine approved for children and adults. KM Biologics is periodically manufacturing LC16 vaccine. There are no issues on the production functions, facilities, and experts to keep the quality and capacity of LC16 vaccine.

In the clinical trial being conducted in Japan, safety data is being collected for high-risk subjects living with HIV. The limited preliminary data has been published. Clinical efficacy studies are ongoing or in preparation to evaluate vaccine effectiveness against mpox in Japan and Colombia.

MVA-BN vaccinia vaccine – research, licensing, demand and production update

Florian Lienert

Bavarian Nordic

The approved shelf life of MVA-BN has been harmonized across the countries in which the vaccine is approved (3 years if stored at -20°C; 5 years if stored -50°C; 9 years if stored -80°C). Regulatory filings of MVA-BN are ongoing in several additional countries.

A NIH-sponsored phase 2 trial comparing the immunogenicity and safety of MVA-BN in adolescents aged 12-17 years to adults aged 18-50 years is ongoing.

Bavarian Nordic has delivered more than 14 million doses of MVA-BN from May 2022 – September 2023. >70 countries have access to MVA-BN, including countries in Latin America and the Caribbean through an agreement with the Pan American Health Organization (PAHO).

Bavarian Nordic has identified a way to scale up the production capacity of MVA-BN by 6 to 50 times (depending on the size of the fermenter used), by developing production in a cell line. Feasibility of the process has been demonstrated and potential timelines for regulatory approval are currently being looked at.

Several reports of the real-world safety profile of MVA-BN have been published, with the largest one reporting surveillance data from the US during a period in which ~1 million MVA-BN doses were administered. In this analysis, the most common adverse health events reported were nonserious and included injection site reactions. Serious adverse events were rare and the vaccine safety profile was consistent with prelicensure studies.

More than 10 reports on the effectiveness of MVA-BN in preventing mpox are now available. The estimated vaccine effectiveness was found to be 36%-89% after 1 dose and 66%-90% after 2 doses. A correlate of protection from mpox disease has not yet been defined. Protection induced by MVA-BN does not correlate with antibody response but might be mainly mediated by T cells.
Regulatory status of antivirals for smallpox and mpox: US FDA perspective

Patrick Harrington

Division of Antivirals, U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Office of New Drugs, Office of Infectious Diseases

Tecovirimat (TPOXX) and brincidofovir (Tembexa) are two small molecule antiviral drugs that are approved by the U.S. FDA for the treatment of smallpox. These drugs were approved based on the FDA’s “Animal Rule” regulations, which provide a pathway for approval of certain drugs and biological products when it is not ethical or feasible to conduct efficacy studies in humans. FDA continues to support and encourage development of additional medical countermeasures for smallpox, particularly for products with different mechanisms of action, different drug interaction and safety profiles, and nonoverlapping drug resistance with tecovirimat and brincidofovir. Tecovirimat and brincidofovir are not FDA approved for the treatment of mpox. FDA has determined that the Animal Rule is not a viable regulatory pathway to approve drugs for the treatment of mpox, as it is both feasible and ethical to conduct clinical trials in humans. In the U.S., tecovirimat is available for the treatment of mpox through a randomized, controlled clinical trial sponsored by the U.S. National Institute of Allergy and Infectious Diseases (https://clinicaltrials.gov/ct2/show/NCT05534984). Tecovirimat is also available under an expanded access protocol sponsored by the U.S. Centers for Disease Control. Brincidofovir is available via single-patient emergency use Investigational New Drug Applications for patients who meet specific eligibility criteria. Information on tecovirimat and brincidofovir and how to access these drugs in the U.S. for the treatment of mpox is provided on the FDA Mpox Response webpage: https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/fda-mpox-response.
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