WHO Advisory Committee on Variola Virus Research

Report of the twenty-fourth meeting, Geneva, 29–30 November 2022

EPIDEMIC AND PANDEMIC PREVENTION AND PREPAREDNESS

World Health Organization

WHO Advisory Committee on Variola Virus Research

Report of the twenty-fourth meeting, Geneva,
29–30 November 2022

World Health Organization

WHO Advisory Committee on Variola Virus Research: report of the twenty-fourth meeting, Geneva, 29–30 November 2022

ISBN 978-92-4-007631-0 (electronic version)
ISBN 978-92-4-007632-7 (print version)

Suggested citation. WHO Advisory Committee on Variola Virus Research: report of the twenty-fourth meeting, Geneva, 29–30 November 2022. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at http://apps.who.int/iris.

This publication contains the collective views of the twenty-fourth meeting of the WHO Advisory Committee on Variola virus Research and does not necessarily represent the decisions or the policies of WHO.

Contents

Abbreviations

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

Executive summary

The World Health Organization (WHO) Advisory Committee on variola virus Research (ACVVR), hereafter referred to as the Committee held its twenty-fourth meeting on 29–30 November 2022, in-person and by video conference. The recommendations of the Committee are summarized in this report.

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. Eighty-eight isolates remained to be sequenced at VECTOR. At CDC, sequencing and analysis were underway in 2022 for 40 additional original isolates. The COVID-19 pandemic and global mpox outbreaks had caused strain on both centres which had hampered sequencing efforts. Both WHO collaborating centres indicated the remaining strains could be sequenced within the next 12 to 24 months. The Committee recommended continuation of previously approved projects.

Antiviral agents

Three compounds were now approved for the treatment of smallpox: tecovirimat, brincidofovir, and NIOCH-14. Oral tecovirimat was approved for treatment of smallpox by the United States Food and Drug Administration (US FDA), Health Canada, the European Medicines Agency (EMA) and the Medicines and healthcare products regulatory agency (MHRA) of the United Kingdom of Great Britain and Northern Ireland. In May 2022, the US FDA approved the intravenous formulation of tecovirimat for treatment of smallpox; development of paediatric formulations continued. Due to the global monkeypox (mpox) outbreak, demand for tecovirimat had risen and randomized controlled trials were underway. The antiviral agent brincidofovir was approved in the USA in tablet and oral suspension formulations for treatment of smallpox in adults and children. Trials of the antiviral NIOCH-14 carried out at VECTOR showed that assessed oral regimens were safe and bioavailable after single and multiple doses. On 4 October 2022, NIOCH-14 was licensed in the Russian Federation for treatment of smallpox, mpox, and cowpox. Testing chemical compounds with high selectivity indices against variola virus *in vitro* would continue. CDC proposed continuing study of a humanized mouse model for assessing smallpox therapeutics. Both WHO collaborating centres continued to explore monoclonal antibodies (mAbs).

Vaccines

Studies with modified vaccinia Ankara vaccine developed by Bavarian Nordic (MVA-BN) continued in different contexts, including for the prevention of human mpox in the Democratic Republic of the Congo; the vaccine had shown an excellent safety profile in health workers and a booster dose study was proposed. Several jurisdictions had extended regulatory indications

for the vaccine to include prevention of mpox and other orthopoxvirus infections for persons at risk. Clinical trials at VECTOR of VACΔ6, a fourth-generation attenuated vaccinia vaccine, showed that it demonstrated lower reactogenicity while retaining immunogenic properties. On 11 November 2022, the vaccine was licensed in the Russian Federation as OrthopoxVac, for immunization against smallpox, mpox, and other orthopoxviruses.

Diagnostics

In 2017 and 2022, four kits for detection and differentiation of pathogenic orthopoxviruses developed at VECTOR were licensed. CDC continued to improve both nucleic acid-based and protein-based rapid diagnostic tests; including novel technologies for othopoxvirus using Loop Mediated Isothermal Amplification which showed promising results. The Committee commended the work of WHO in advancing the mpox diagnostics roadmap and providing countries with diagnostics support during the global mpox outbreak. The Committee considered advances made regarding the development of mpox diagnostics during the global outbreak which included WHO interim guidance for laboratory testing for Monkeypox virus, development of target product profiles for mpox rapid diagnostics, and an external quality assurance (EQA) scheme for Monkeypox virus testing.

Lessons learned from the multi-country mpox outbreak

The Committee discussed the implications for preparedness for smallpox-like events reflected by the ongoing global mpox outbreak. The Committee noted how quickly medical countermeasures such as diagnostics, therapeutics and vaccines could be deployed when resources and political will were abundant. The extensive research on variola virus countermeasures had been leveraged to respond to the mpox outbreak. The strengthening of laboratory and genomic sequencing capacity during the COVID-19 pandemic were leveraged to scale up testing for mpox. Third generation smallpox/mpox vaccines were made available to immunize high risk groups during the mpox outbreak in many countries through a range of procurement mechanisms. Tecovirimat was made available in a few settings through expanded access programmes, randomized controlled trials and and deployment of limited WHO reserves for compassionate use. The Committee resolved that more work was needed to understand modes of transmission between people and identify animal reservoirs of the Monkeypox virus and to enhance access to countermeasures developed.

Conclusion

The Committee noted that with the approval of a third antiviral and fourth generation vaccine, the original objectives of the research programme endorsed by the World Health Assembly (WHA) were being met. Signals of resistance to tecovirimat in a few cases of mpox were concerning, suggesting that continued development of additional therapeutics with different mechanisms of action may be warranted. The Committee also noted that WHO should consider replenishing emergency vaccine reserves. It was noted that implementation of the roadmap for development of diagnostic assays for mpox had been accelerated during the outbreak.

Countermeasures developed for smallpox preparedness had been key in responding to the global mpox outbreak, illustrating the public health benefit of the variola virus research programme. Lessons learned from the mpox outbreak continue to inspire development efforts for rapid, point-of-care diagnostics. The Committee reaffirmed the importance of continued development of protein-based assays for orthopoxviruses and recommended that target product profiles be developed for smallpox diagnostics. Other recommendations offered by the Committee are summarized in the report.

Meeting proceedings

The twenty-fourth 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, 29–30 November 2022. The meeting was chaired by Dr David Ulaeto. The agenda is included as Annex 1 and the list of participants as Annex 2. The current approval status of research proposals from the WHO collaborating centres is presented in Annex 3. Annex 4 includes the abstracts related to approved research proposals. This was a hybrid meeting with in-person and remote participation by video conference.

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 matter 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 discussed at the meeting.

The objectives of the meeting were to:

- review progress of approved research with live variola virus (VARV);
- review the research programme and recommendations for 2020-2022; and
- develop the research agenda for 2023

Dr Michael Ryan, Executive Director, WHO Health Emergencies Programme welcomed all participants and provided opening remarks. He discussed the importance of this committee not just for smallpox preparedness but noted the relevance of its work in responding to the multi-country mpox outbreak that emerged this past year. He re-emphasized the importance of reflecting on the lessons from COVID-19 and mpox. In particular, the mpox outbreak had demonstrated the threat orthopoxviruses (OPXV) present to the global community highlighting the work this Committee did and its value for ongoing development of medical countermeasures and preparedness.

Dr David Ulaeto, chairperson, introduced the agenda: updates from the two WHO collaborating centres on their variola virus and DNA collections as well as on the progress of research. There would also be discussion of the roadmap for mpox diagnostics requested the previous year, and presentations from the WHO Secretariat and partners. He reminded Committee members that the focus of the Committee[1] was to oversee research on vaccines, antivirals and diagnostics as well as advising on biosafety inspections of the repositories and the timing of destruction of variola virus stocks. Considering the global mpox outbreak and COVID-19 pandemic, he requested that Committee members consider the original goals and how to proceed with achieving them. There were no objections to the agenda for the meeting from other Committee members.

1 Terms of reference can be found at: https://www.who.int/docs/default-source/documents/health-topics/smallpox/tors-acvvr.pdf

Secretariat report

Dr Rosamund Lewis, Head of the Smallpox Secretariat, WHO Health Emergencies Programme, welcomed new members and all participants, took roll call and thanked members who had left in 2022: Professor Tamfun Muyembe and Dr Zalini Binti Yunus. 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 for 2020–2022 previously agreed with the United States Centers for Disease Control and Prevention (CDC) and the State Research Center of Virology and Biotechnology (VECTOR) following the Seventy-second World Health Assembly and endorsed by WHO at successive meetings was published in the form of a three-year roadmap in previous Comittee reports. A brief overview of the programme was provided. Ongoing research would be presented as progress reports, as the the global mpox outbreak and building renovations had affected completion of approved projects.

An update was presented on orthopoxvirus events over the past year. Another case of Alaskapoxvirus infection was reported in 2022, bringing the number of human infections with this newly described orthopoxvirus to five. Previous cases had occurred in 2015 (one case), 2020 (one case), and 2021 (two cases), all in the state of Alaska in the United States of America. On 7 May 2022, WHO received a report of monkeypox from the United Kingdom of Great Britain and Northern Ireland in a traveller from Nigeria, for which no secondary cases were identified. This was the first such imported case reported in a woman. WHO published a Disease Outbreak News report for this case.[3]

Dr Lewis then provided an overview of the global outbreak of mpox, which began in 2022. On 13 May 2022, WHO received a report of mpox in a 9-day old infant from a family with no travel links to Nigeria, which led to discovery of a family cluster of three cases.[4] The investigation rapidly led to discovery of many undiagnosed cases and rapid evolution of an outbreak in many countries, ultimately in all six WHO regions. Numerous mpox cases and clusters had been concurrently reported from several countries outside of the usual ecological niche for mpox in widely disparate geographic areas. Most cases had been reported through sexual health or other health services in primary or secondary-health care facilities and had involved mainly but not exclusively men who have sex with men. Due to the extraordinary nature of the event, rapid international spread of the outbreak in countries that had not previously seen the disease with a risk to human health, and the need for international coordination, the Director General of WHO convened an Emergency Committee under the International Health Regulations (IHR),[5] and declared the multi-country outbreak a Public Health Emergency of International Concern (PHEIC) on 23 July 2022.[6]

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

3 Disease Outbreak News Monkeypox – United Kingdom of Great Brian and Northern Ireland. Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON381

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

6 Second meeting of the International Health Regulations (2005) (IHR) Emergency Committee regarding the multi-country outbreak of monkeypox. Available at: https://www.who.int/news/item/23-07-2022-second-meeting-of-the-international-health-regulations-(2005)-(ihr)-emergency-committee-regarding-the-multi-country-outbreak-of-monkeypox

As of 26 November 2022, there were 81,107 confirmed cases and 55 deaths reported from 110 countries.[7]

The multi-country outbreak and declaration of a PHEIC had accelerated the work for mpox, for which WHO provided extensive support including interim guidance on the following:

1. surveillance, case investigation and contact-tracing for mpox;[8] establishment of a global surveillance platform and case-reporting tools; and update of the mpox outbreak toolkit;[9]
2. laboratory testing for the Monkeypox virus; and provision of support for enhancing PCR testing capacity at national and subnational levels,[10] expansion of genomic sequencing capability to understand virus evolution, quality assessment and assurance for diagnostic assays,[11] and establishment of target product profiles for rapid diagnostic tests;[12]
3. clinical management and infection prevention and control;[13] and supporting access to treatment through a core protocol for clinical trials,[14] designing expanded access options,[15] and deployment of a limited WHO reserve for compassionate use to requesting countries;
4. vaccines and immunization for mpox,[16] supporting vaccine procurement in the Americas,[17] coordinating with partner agencies to avail supply to requesting Member States, and working with manufacturers on emergency review of their product files to WHO;
5. risk communication and community engagement for mpox outbreaks;[18] and
6. publication of a large suite of public health advice documents for affected populations.[19]

---

7 At the time of publication, over 87 000 confirmed and over 1000 probable cases of mpox and over 140 deaths had been reported from 111 countries. These figures do not include suspected cases of mpox or deaths linked with suspected cases. All data are available on the platform for the global mpox outbreak. Available at:at: https://worldhealthorg.shinyapps.io/mpx_global/

8 Surveillance, case investigation and contact tracing for mpox (monkeypox): interim guidance, 22 December 2022, Available at: https://www.who.int/publications/i/item/WHO-MPX-Surveillance-2022.4

9 WHO mpox outbreak toolbox. June 2022. Available at: https://www.who.int/emergencies/outbreak-toolkit/disease-outbreak-toolboxes/mpox-outbreak-toolbox

10 Laboratory testing for the *Monkeypox virus*: Interim guidance. Available at: https://www.who.int/publications/i/item/WHO-MPX-laboratory-2022.1

11 J. Michel and al; Evaluation of 11 commercially available PCR kits for the detection of *Monkeypox virus* DNA, Berlin, July to September 2022. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9650706/

12 Public consultation: Target Product Profiles for tests used for diagnosis of mpox (monkeypox). Available at: https://www.who.int/news-room/articles-detail/public-consultation--target-product-profiles-for-tests-used-for-diagnosis-of-mpox-(monkeypox)

13 Clinical management and infection prevention and control for monkeypox: Interim rapid response guidance. Available at: https://www.who.int/publications/i/item/WHO-MPX-Clinical-and-IPC-2022.1

14 CORE PROTOCOL - An international adaptive multi-country randomized,placebo-controlled, double-blinded trial of the safety and efficacy of treatments for patients with *Monkeypox*. Available at: https://www.who.int/publications/m/item/core-protocol---an-international-adaptive-multi-country-randomized-placebo-controlled--double-blinded-trial-of-the-safety-and-efficacy-of-treatments-for-patients-with-monkeypox-virus-disease

15 Emergency use of unproven clinical interventions outside clinical trials: ethical considerations. Available at: https://www.who.int/publications/i/item/9789240041745

16 Vaccines and immunization for monkeypox: Interim guidance, 16 November 2022. Available at: https://www.who.int/publications/i/item/WHO-MPX-Immunization

17 https://www.paho.org/en/news/5-8-2022-countries-approve-resolution-support-access-monkeypox-vaccine-americas

18 Risk communication and community engagement (RCCE) for monkeypox outbreaks: Interim guidance, 24 June 2022. Available at: https://www.who.int/publications/i/item/WHO-MPX-RCCE-2022.1

19 https://www.who.int/news-room/questions-and-answers/item/monkeypox; https://www.who.int/news-room/public-advice; Advice for health workers file:///C:/Users/lewisr/Downloads/update_may22_Monkeypox.pdf; https://www.who.int/publications/m/item/public-health-advice-on-mpox-and-congregate-settings--settings-in-which-people-live--stay-or-work-in-proximity; https://www.who.int/publications/m/item/public-health-advice-for-sex-workers-on-monkeypox; and https://www.who.int/publications/m/item/public-health-advice-on-mpox-(monkeypox)-and-sex-on-premises-venues-and-events

During the outbreak, community organizations, scientists and some governments advocated for new nomenclature for monkeypoxvirus (MPXV) clades and for the infectious disease caused by them. In August 2022, WHO convened an ad hoc expert meeting of orthopoxvirologists including members of this Committee, evolutionary biologists and other scientists to discuss characteristics of MPXV clades and propose names for them. Consensus was reached for nomenclature using a Roman numeral for each clade with lower-case Latin characters for sub-clades; the Congo Basin clade became Clade I and the West African clade Clade II, encompassing two phylogenetically distinct subclades IIa and IIb. In November 2022, following an accelerated process under the International Classification of Diseases, WHO introduced the term mpox as a synonym for monkeypox, which would be effective in ICD-11 as of January 2023.[20]

Other novel findings and developments during this outbreak included:

7. outbreaks began in Europe, spread to North America and to Central and South America and other parts of the world;[21]
8. further elucidation of MPXV clades and strains along with implications
    a. clade I may be more widespread than previously known, including identification of outbreaks in new locations (Sudan)[22] and contexts (e.g. refugee camps);[23]
    b. clade II infections during the global outbreak had primarily resulted from person-to-person transmission, with clade IIb.B.1, the leading outbreak lineage, never having been identified in animals;
9. improved clinical characterization of mpox;[24]
10. advances in research and regulatory processes for therapeutics and vaccines.

Global preparedness of WHO Member States for this unprecedented outbreak was supported by the work of this Committee on vaccines, therapeutics, and diagnostics, as well as rapid WHO response under the health emergency programme incident management procedures and existing platforms adapted from the COVID-19 response, and availability of online resources and training materials for mpox as prepared over the last few years. Nonetheless, while the public health value of research for smallpox preparedness was highlighted by the response to mpox, the continuing inequity of access to countermeasures within and between countries remained an unresolved issue in this outbreak.

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 at the CDC was conducted in May 2022; the inspection of facilities and procedures at VECTOR was rescheduled to 2023.

---

20 WHO recommends new name for monkeypox disease; Please see: https://www.who.int/news/item/28-11-2022-who-recommends-new-name-for-monkeypox-disease; Please also see: D. Ulaeto et al, New nomenclature for mpox (monkeypox) and *Monkeypox virus* clades. Available at: https://doi.org/10.1016/S1473-3099(23)00055-5

21 2022-23 Mpox outbreak: Global Trends. Available at: https://worldhealthorg.shinyapps.io/mpx_global/#1_Overview

22 Mediterranean Regional office, weekly epidemiological monitor. Available at: https://applications.emro.who.int/docs/EPI/2022/2224-4220-2022-1531-eng.pdf?ua=1

23 Multi-country outbreak of mpox, External situation report #16 - 16 February 2023. Available at: https://www.who.int/publications/m/item/multi-country-outbreak-of-mpox--external-situation-report--16---16-february-2023

24 22-23 Mpox outbreak: Global Trends. Available at: https://worldhealthorg.shinyapps.io/mpx_global/#33_Case_profile_(overall)

Ms Alexandra Hill, Technical Officer, Health Emergencies Programme, also shared an update on the inspection of the WHO smallpox vaccine emergency reserve of 2.8 million doses of mainly first-generation smallpox vaccine, conducted in January 2022. The inventory of physical stock was performed on 26 January 2022, with sample vaccines taken for potency testing at the WHO collaborating centre on smallpox vaccine, the Netherlands (Kingdom of the) National Insitute for Public Health and the Environment (RIVM). Their findings are provided later in this report.

In 2022, prior to the global mpox outbreak, WHO had initiated procurement of tecovirimat for proof-of-concept for small scale *ad hoc* requirements. When the mpox outbreak began, the reserve was made available to WHO regions for compassionate use for mpox, a facility accessed by two countries (the Federative Republic of Brazil and the Republic of Chile). Additionally, SIGA Technologies donated 2500 treatment courses of tecovirimat to WHO to be provided under an expanded access protocol (EAP).[25] Discussions on donations of third-generation vaccines from the government of Japan and from the European Health Emergency Preparedness Response Authority (HERA) were on going.

Dr Lewis reviewed the WHO workplan for 2023 which, to ensure that public health benefit should continue to accrue from the work of the Committee, included:

- continuing policy and technical support for the global mpox response;
- continuing engagement with the WHO Strategic Advisory Group of Experts on Immunization (SAGE) to update recommendations on use of smallpox vaccines;
- continuing work on countermeasure reserves, protocols, guidelines and engagement with Member States including striving for equity;
- completing the current cycle of repository inspections,
- continuing countermeasures research and development; and
- preparing for discussions on smallpox preparedness and research at the Seventy-seventh World Health Assembly in 2024.

25 Protocol for Monitored Emergency Use of Unregistered and Experimental Interventions (MEURI). Challenges experienced by countries in accessing supplies under MEURI included varying perception of risk and need, lack of awareness of procedures, short windows for completion of expressions of interest, and underestimation of the time required to complete confidentiality agreements and ethics review. At the time of publication, no treatment doses have been provided through this mechanism.

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 Rinat Maksyutov shared an update on the variola virus collection at the State Research Center of Virology and Biotechnology, Rospotrebnadzor (VECTOR). The variola virus research laboratories at VECTOR were in compliance with national and international requirements and with WHO recommendations from successive biosafety and biosecurity inspections. The variola virus collection comprises 120 strains (primarily unique strains), with no change in the collection since the last report in 2021. Eighty-eight isolates remain to be sequenced.

The projects requiring use of variola virus approved by WHO to be conducted in 2022 were:

1. replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development (Approved 2020);
2. assessment of the neutralizing activity of vaccinated volunteers' sera and those of vaccinated animals using variola virus to support the development of less-reactogenic fourth generation smallpox vaccines (Approved 2019);
3. discovery and testing of novel chemical antivirals for smallpox treatment and prevention (Approved 2019);
4. evaluation of antivirals against smallpox based on monoclonal antibodies (Approved 2019); and
5. development of advanced methods for rapid (point-of-care) diagnostics of orthopoxvirus infections (Approved 2020).

Progress reports would be presented. Regarding replenishment of stocks with non-infectious material derived from live variola virus for diagnostic purposes, no sequencing activity was performed in 2022. Hence, isolation of 50 variola virus DNA samples to conduct whole genome analysis would be undertaken in 2023.

Dr Maksyutov reported that the fourth-generation smallpox vaccine OrthopoxVac, the antiviral NIOCH-14, and four different kits to detect and differentiate orthopoxviruses in humans including mpox were licensed by the Ministry of Health of the Russian Federation in 2017 and 2022. With respect to use of live VARV, Dr Maksyutov reported that virus was used for studies of the neutralizing capacity of sera from studies of vaccinia variants in development of fourth-generation vaccines. Following continuing WHO endorsement of previously approved projects proposed by VECTOR, they were scheduled to resume in 2023.

Report on the variola virus collection at the WHO Collaborating Centre for Smallpox and Other Poxviruses – United States Centers for Disease Control and Prevention, USA

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 in compliance with national and international requirements and with WHO recommendations. WHO completed inspection of the variola virus repository and research laboratory at CDC in May 2022.[26] CDC had completed initial assembly and phylogenetic analysis of all isolates with extracted DNA to date. 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.[27] DNA extraction was completed in June 2022 for these additional isolates, following established protocols, and sequencing and analysis were underway.

The projects requiring use of live variola virus approved by WHO for conduct in 2022 were to:

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

In total, variola virus had been used 60 times: 19 uses for nucleic acid-based diagnostic assays; 15 uses for smallpox small animal model development; 16 uses for antiviral therapeutic development (tecovirimat) and 10 uses to evaluate the efficacy of other potential antiviral compounds (monoclonal antibodies (mAbs)).

26 WHO *variola virus* repository and collaborating centre inspection reports are available at: https://www.who.int/activities/variola-virus-repository-safety-inspectionsy safety inspections (who.int)

27 Another 70 or so isolates had been used to completion in various research projects in the past.

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.

In line with previous recommendations of the Committee, direct sequencing of clinical specimens and virus stocks had been established, reducing the need for virus propagation and purification prior to sequencing (thereby reducing work with live virus). So far sequencing at CDC had been performed on specimens with sufficient epidemiological metadata for epidemiological analysis. With new work delayed by the global monkeypox outbreak, it was estimated that sequencing of remaining isolates without metadata could be accomplished in 2023. VECTOR had indicated that of 88 remaining specimens to be sequenced, 50 could be completed in 2023. It had previously been noted that WHO collaborating centres were encouraged to prioritize specimens for sequencing, and to extract DNA from all unsequenced specimens to facilitate later sequencing.

Research reports and proposals

Antivirals

VECTOR: clinical trials on the anti-smallpox drug NIOCH-14

Dr Alexander Agafonov shared an update on the drug NIOCH-14, named for the N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry where the compound was made. Following previously reported clinical studies conducted to assess safety, tolerability and pharmokinetics of the drug, a Phase I trial approved in July 2020 had begun in September of the same year and enrolled 90 persons (aged 18–50 years). Six groups of 15 participants each received various dose regimens as single doses for one day (200 mg, 600 mg and 1 200 mg) or daily doses for a period of six days each of 200 mg, 600 mg and 1 200 mg (split into two doses).

Safety assessment had been conducted on a fixed schedule on six occasions from 3 to 90 days after drug administration. With single daily doses, and with six-day regimens of 200 or 600 mg daily, no adverse events had been reported. One volunteer receiving the 1 200 mg daily regimen had reported chest and abdominal pain on days 2 and 5, which was not linked to any specific diagnosis or change in clinical parameters. Clinical assays had not shown any damage to the liver, kidney, heart or pancreas. Thus the Phase I clinical trial, as previously reported, had demonstrated that the smallpo,x antiviral drug NIOCH-14 (in the form of hard gelatin capsules containing 200 mg of NIOCH-14 and excipients) was bioavailable and safe when administered orally in single-dose or six-day regimens to volunteers.

In 2022, the antiviral efficacy of NIOCH-14 was evaluated against a new strain of MPXV, St Petersburg-22, a strain isolated from a sick patient in July 2022. The inhibitory concentration (IC50) of NIOCH-14 for MPXV St. Petersburg-22 was 0.02 µg/ml and the selectivity index was > 15 000. This demonstrated that NIOCH-14 exhibits high *in vitro* antiviral activity against against the new MPXV strain isolated 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, monkeypox and cowpox for persons aged 18 -50 years (600 mg twice daily for 14 days). There were plans for 2023 to obtain permanent marketing authorization for NIOCH-14.

VECTOR: discover and test novel chemical antivirals for smallpox treatment and prevention

Dr Agafonov shared the ongoing research of novel chemical antivirals for smallpox treatment (project approved in 2019). The goal of this study had been to test the antiviral activity of novel chemical compounds using surrogate orthopoxviruses to identify the most effective drugs for further efficacy assessment against live variola virus *in vitro*. Between 2019 and 2022, more than 500 new compounds of different classes had been tested. Orthopoxviruses studied were *Vaccinia virus* (Copenhagen strain), *Cowpox virus*

(Grishak strain) and *Ectromelia virus* (K-1 strain). Cidofovir (Heritage Consumer Products, LLC, USA) and ST-246[28] were used as reference compounds.

Among the tested compounds, 25 compounds were identified whose selectivity indices (SI) against the surrogate orthopoxviruses (*Vaccinia virus*, *Cowpoxvirus* and *Ectromelia virus*) ranged from 100 to 18 100. The most active compounds against surrogate orthopoxviruses were chemical structures containing fragments of monoterpenes, camphor, borneol, adamantane, isobornylamine, phenylamides, benzylamides, bicyclic backbone, and 2-aryl-1-hydroxyimidazole derivatives. These compounds would be tested using live variola virus in a project proposed to be conducted in 2023.

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

Dr Todd Smith presented an update to the research project 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 ten identified F13L amino acid variants tested so far were sensitive to tecovirimat. The remaining two F13L variants would be expressed in stable cell lines to evaluate sensitivity to tecovirimat during infection with *Vaccinia virus* not expressing the F13L protein (VACVΔF13L).

An update was provided on the use of tecovirimat in the context of the multi-country mpox outbreak. Under an expanded access protocol, 5 764 patients have received tecovirimat for the treatment of mpox in the USA. The US FDA released additional information on tecovirimat resistant genotypes which had been confirmed in at least two cases. In both cases, the patients were severely immunocompromised and had received extended tecovirimat treatment. In one case, the isolate was completely resistant to tecovirimat and the other had partial resistance.

In 2023, plans for testing with tecovirimat include analysis of newly sequenced isolates to determine if additional F13L variants exist and if necessary, to create stable cell lines expressing the variola virus F13L protein for evaluation of sensitivity during infection with VACVΔF13L.

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 sensitive, and two ST-357 analogues were screened against a *Vaccinia virus* Western Reserve strain. Since 2020, due to challenges encountered with solubility characteristics of ST-357, this project had been delayed until additional analogues become available to assess.

28 This compound (tecovirimat) was synthesized for research purposes by NIOCH SB RAS, the Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Science, according to a described process.

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

Dr Todd Smith presented an update on this project (initial findings previously published).[29] Two strains of humanized mice had been found to be highly susceptible to variola virus and suitable for study as an animal model for smallpox. To validate the model for testing new smallpox therapeutics, Hu-CD34 and BLT mice were infected with variola virus, showing systemic infection around days 9 to 10 post-infection, and treated with tecovirimat. As previously reported, validation in hu-CD34 mice treated at 0, 1 or 2 days post-variola virus challenge had shown some protection for tecovirimat treated groups compared to untreated controls, particularly when administered early; the group with tecovirimat administered on day 0 of virus challenge had 86% survival, median survival without treatment was 16 days, with 50% mortality appearing with later treatment. Earlier treatment initiation with tecovirimat was associated with lower virus titres, especially in secondary sites (nose, skin, tongue). All mice in the group without tecovirimat treatment succumbed to infection. There were 3 animals that were variola virus negative by PCR and excluded from the survival analysis and euthanized at days 5, 7 and 12 post-infection for weight loss that had no significant pathology to explain this and 2 animals had virus neutralizing antibodies. There were 3 animals that succumbed to variola virus with extensive secondary bacterial infection who succumbed or were euthanized on day 19, 32 and 33 respectively post infection indicating the risk of secondary infection which was also common in smallpox patients. Additionally, 7 animals had necrosis of the adrenal gland which has been witnessed in patients in mpox indicating this was an authentic model and may mimic what happens with other orthopoxviruses. Repeat studies with the more immunocompetent hu-BLT mice approved by WHO in December 2021 were planned to be completed in January 2023.

Preliminary results from studies to identify early biomarkers of variola virus infection showed the highest activated genes were for inflammatory markers IL-8 and IFIT2. Many markers involved in B-cell regulation (reflecting potential antibody production) had been down-regulated and the two most down regulated genes were MS4A1 and BLNK; the gene for CCL7, a marker for monocyte attraction, was the only gene with significant and relevant activation across time. Continuation of biomarker analysis on samples from prior studies was planned for 2023.

Tecovirimat and ST-357: licensing and product update

Dr Dennis Hruby provided further updates on tecovirimat and ST-357. 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 December 2021.[30] It was also 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 UK Medicines and Healthcare products Regulatory Agency (MHRA) in June 2022. The USA product shelf life of the oral formulation is currently set at 7 years. Continuing development for oral tecovirimat was reported to include: i) pharmacokinetic clinical studies for powder formulations for

29 This project had been partially approved in 2019 and fully approved in 2020, with an extension approved in 2021.
30 Tecovirimat was approved on 1 December 2021 by Health Canada for the treatment of smallpox and on 10 January 2022 by the European Medicines Agency. Marketing authorizations were approved as requested and outlined in the text.

children < 13 kg; ii) studies of tecovirimat for post-exposure prophylaxis (with the United States Department of Defense), and iii) the impact of tecovirimat on the efficacy of MVA-BN vaccine use in post-exposure vaccination.

In May 2022, an intravenous (IV) formulation of tecovirimat with a shelf life of 24 months was approved by the US FDA for treatment of smallpox in adults and paediatric patients weighing ≥ 3 kg, which had also been used off-label during the mpox outbreak in 2022. Further tests were ongoing to evaluate the integrity of the intravenous delivery mechanism. Guidance for switching from intravenous to oral tecovirimat was being developed.

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 and was supporting eight observational and randomized controlled clinical trials (RCTs) to collect safety and efficacy data and further characterize mpox.

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

Brincidofovir: licensing and production update

Dr Laura Cochrane provided an update on the licensing and production of brincidofovir. Emergent Biosolutions now produces brincidofovir from bulk drug substance supplied by Chimerix. Brincidofovir had a different mechanism of action than tecovirimat or NIOCH-14 and a relatively high barrier to development of resistance. Drug substance and drug product manufacturing processes for the tablet and suspension had been validated. In June 2021, the US FDA approved the short-course therapeutic in tablet and oral suspension formulations for the 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); ongoing stability monitoring is planned to explore extending the shelf life. Brincidofovir had been added to the US strategic national stockpile and Canada has acquired it under a special access program.

The global mpox outbreak had led countries to request procurement of brincidofovir and Emergent Biosolutions is developing manufacturing plans for 2023. It is currently available for treatment of mpox in the USA under an investigational new drug (IND) request. US FDA plans *in vitro* evaluation of brincidofovir against various strains of orthopoxviruses, pharmacokinetics bioequivalence studies, and support of clinical investigations as appropriate.

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. Tecovirimat and brincidofovir had been approved based on the “animal rule” for smallpox disease.

For mpox, tecovirimat was reported to be available in the USA through a RCT or under an EAP. Brincidofovir was available through an emergency use IND application. Cidofovir

was another antiviral which had been used off-label for mpox and other orthopoxvirus infections and should be used through a randomized controlled clinical trial (RCT) to establish efficacy and safety.

Dr Lorna Leal Alexander provided an update on the regulatory status of antivirals on behalf of the European Medicines Agency (EMA). Tecovirimat was approved on 6 January 2022 for the treatment of smallpox, mpox and *Vaccinia virus* infection, based on *in-vitro* and *in-vivo* nonclinical studies. Brincidofovir was granted orphan designation for the treatment of smallpox disease and cidofovir was withdrawn from use in the European Union at the request of the marketing authorisation holder but is still available in several EU (EU) Member States. While vaccinia immune globulin (VIG) is available in other countries, it is not approved by the EU for use in any orthopoxvirus infection. Several randomized RCTs were ongoing to evaluate the safety and efficacy of tecovirimat and find the best treatment for patients with mpox.

Monoclonal antibodies

Evaluate antivirals against smallpox based on monoclonal antibodies

Professor Sergei Shchelkunov 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 obtained mAbs for selected target proteins; and iv) evaluating the neutralizing properties of mAbs on *Vaccinia virus*. As a result, cell line producers of OPXV immunodominant proteins and CHO-K1 cell lines for production of recombinant antibodies had been developed. Three OPXV-specific human mAbs and a chimeric mAb M12B9ch specific for the L1 protein were produced.

A plaque reduction neutralization test ($PRNT_{50}$) of *Vaccinia virus* (LIVP strain) was used to assess the neutralizing activity of mAbs in cell culture. All three human mAbs neutralized *Vaccinia virus* at a concentration of 1.5–7.6 µg/mL, while the chimeric antibody M12B9ch neutralized the virus at a concentration of 0.125 µg/mL, all in the absence of complement. When the M12B9ch antibody was evaluated in the presence of human complement (2.5%), neutralizing activity at a concentration of 0.002 µg/mL was shown. A patent for the M12B9ch antibody in the Russian Federation was expected imminently.[31] Monoclonal antibodies would be further tested for antiviral properties and neutralizing activity against VARV, MPXV, other OPXVs and a search for new variants of human antibodies capable of neutralizing OPXVs would continue.

31 On 12 January 2023, a patent of the Russian Federation was granted for recombinant plasmid vector pVEAL-M12B9ch providing stable expression of chimeric monoclonal antibody M12B9ch against orthopoxviruses in mammalian cells and recombinant chimeric monoclonal scFv-Fc antibody.

Use of live variola virus to evaluate antivirals (monoclonal biologics) against variola virus (Approved 2019)

Ms Audrey Matheny shared an update on development of monoclonal antibodies. Due to concerns about the resistance of variola virus to tecovirimat and considering that a multi-therapeutic approach to smallpox treatment would be desirable, mAbs and mixes of mAbs are being researched. As previously reported, CDC was working with several partners on this.

In 2021, CDC tested newly produced individual mAb candidates from Vanderbilt University against variola and MPXV. Based on the results, four mixes were designed and tested against the intracellular mature virion (IMV) and enveloped virion (EV) forms of both viruses; all mixes appeared to be effective against variola virus and Monkeypox virus. In 2022, individual mAbs and four mix combinations administered one day pre-challenge protected mice from severe weight loss, and mice given these treatments survived more often than mice who received others. In 2023, CDC plans to further evaluate the mixes against variola virus and MPXV before determining a final combination of mAbs

In 2021, CDC had also tested humanized and chimeric mAbs from BioFactura. The anti-L1 humanized antibody (h7D11) was found to be non-inferior to chimeric antibody (c7D11) in neutralizing the IMV form of variola virus by PRNT with EC_{50} concentrations of < 0.01 µg/mL, with or without complement. The research team had proposed that larger scale production should continue for both 7D11 and 8A mAbs with *in vitro* neutralization against variola virus continuing at critical production steps. Thus, in 2022, anti-L1 and anti-B5 mAbs and cocktail were tested against VARV. Anti-L1 mAb showed comparable neutralization to the previous production run while anti-B5 mAb showed slightly lower neutralization. The cocktail contained only these two humanized antibodies and showed higher neutralization than the three-mAb chimeric version tested in 2019. EC50 values for the current cocktail against the IMV form of VARV with and without complement were < 0.02 ug/mL, and the EC50 against the EV form was 0.56 ug/mL. Individual mAbs showed slightly higher neutralization than the mix. The mAb and mix neutralization capability was lower against Clade I MPXV (EC50=0.005 – 1.05 ug/mL).

In summary, mAb products from partner entities continued to show promise against variola virus *in vitro* with some variability between production runs.

Discussion on antiviral and animal model research projects

Several observations and recommendations were made.

The Committee noted that licensure of a new antiviral drug (NIOCH-14) was an important accomplishment and that the objectives of research with live VARV were being achieved. It was noted that the need for at least two antiviral agents for smallpox had been discussed by the Seventy-second World Health Assembly as the primary rationale for continuing retention of variola virus stocks and this new development would need to be presented to Member States.

The main concern based on lessons learned from the global mpox outbreak was over reports of tecovirimat resistance-associated viral mutations in two patients with advanced immune suppression who had received prolonged tecovirimat therapy, and what this

could mean in the context of a smallpox outbreak. Aside from potential development of resistance to tecovirimat, NIOCH-14 or brincidofovir, other factors could affect their use, such as immune suppression or interactions with other medications. It was observed that antivirals stop viral replication until the immune system can eliminate the virus; when immune response is limited, longer treatment can generate resistance. It was suggested that other therapeutic options be explored; considerations for optimal duration of therapy and evaluation of drug bioavailabilty to prevent emergence of resistance were discussed. Members recommended using lessons from HIV and Hepatitis C therapy and suggested studies of combination therapy with antivirals having different mechanisms of action. In the USA, multi-medication regimens used in severe mpox sometimes included brincidofovir or cidofovir along with tecovirimat. At this time, as use of tecovirimat in combination therapy had been outside of controlled studies the benefit was unclear; it would also be important to assess adverse reactions.

Another concern raised was whether viral mutations could lead to emergence of more virulent variants. Based on tecovirimat resistant viruses that had been tested in mice, it was found that some variants were less virulent, some were of equal virulence, and some were more virulent. The Committee was of the view that it was unclear what might happen in the event of a smallpox outbreak if drug resistance were to develop. Regarding monoclonal antibodies, while Committee members supported continuing development, concern was expressed that monoclonal antibodies would not in the short term offer solutions for low resource settings due to their cost and the resources needed for administration.

In conclusion, it was agreed that outcomes from public health-relevant research on variola virus did support mpox prevention and control in the 2022 global outbreak. The Committee recognized that while benefits for mpox outbreak response had resulted from work towards preparedness for a smallpox emergency, research on effectiveness of countermeasures for mpox was essential and equity of access remained elusive, even within a framework of public health and clinical studies. Overall the Committee was of the view that research for development of orthopoxvirus therapeutics should continue considering a range of viral targets and demographic groups at risk. Recommendations made on this topic can be found at the end of the report.

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

Professor Sergei Shchelkunov reminded the Committee that preclinical studies on the fourth-generation VACΔ6 vaccine in rabbits, guinea pigs and mice had shown high activity of variola virus-neutralizing antibodies in the blood of vaccinated animals at 42 days and 6 months following vaccination. Following Phase I to III clinical studies, in 2020–2021, a double-blind, comparative, randomized, placebo-controlled trial of VACΔ6 in four parallel groups had been conducted, with 334 volunteers aged 18–60 years enrolled. Participants had received either a single dose of VACΔ6 vaccine

(at 10^7 PFU[32]) or two doses of VACΔ6 (at 10^6 PFU) with a three-week interval between doses, or a one or two dose placebo regimen respectively for comparison.

Studies had demonstrated that two doses of the VACΔ6 vaccine and a single dose of the classical first-generation LIVP-based[33] vaccine licenced in the Russian Federation both induced generation of *Vaccinia virus*-neutralizing antibodies in study participants with no significant difference in the geometric mean antibody titres in the sera of volunteers in both groups. The development of orthopoxvirus specific antibodies following receipt of VACΔ6 vaccine was independent of dose and regimen. Individuals who received single or double vaccination reported localized skin reactions; generalized reactions were not reported. In 2022, the assessment of neutralizing activity against VARV in the sera of volunteers continued in approved studies.

The fourth-generation VACΔ6 cell culture-based vaccine was licensed as OrthopoxVac in the Russian Federation on 11 November 2022. It was recommended for immunization for adults aged 18 to 60 years against smallpox, mpox, and other orthopoxvirus infections. The main advantage of the VACΔ6 vaccine was that it demonstrated lower reactogenicity than the classical vaccine while retaining immunogenic properties. The shelf-life at 2-8° C of the product was 5 years and antibiotics had not been used as preservative agents.

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 Brett Petersen provided an update on the study of vaccination of health workers against mpox using MVA-BN vaccine in Tshuapa province, in the Democratic Republic of the Congo (DRC).[34] 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 1 000 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 among participants during two-year 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 the enzyme-linked immunosorbent assay (ELISA). Participants with presumed prior smallpox vaccination (based on age) had had a more durable antibody response than vaccine-naive 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

32 Plaque-forming unit.

33 Lister-IVP: Lister vaccine adapted by the Institute for Viral Preparations (Moscow) which was used in the Russian Federation as the first generation smallpox vaccine. Shchelkunov SN, Sergeev AA, Yakubitskiy SN, Titova KA, Pyankov SA, Kolosova IV et al. Adaptive immune response to *Vaccinia virus* LIVP infection of BALB/c mice and protection against lethal reinfection with *Cowpox virus*. Viruses. 2021;13(8)1631. doi:10.3390/v13081631.

34 The study was a collaboration between the Ministère de la Santé de la République démocratique du Congo, l'Ecole de Santé publique de Kinshasa, the International Communication and Education Foundation and CDC.

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.

An update was provided on the use of MVA-BN in the USA, where it was licensed for use in persons 18 years of age or older to be administered subcutaneously with an injection volume of 0.5 mL 4 weeks apart. During the mpox outbreak, on 12 August 2022, the US FDA issued Emergency Use Authorization for MVA-BN and provided an alternative regimen which included 0.1 mL intradermal administration 4 weeks apart to persons ≥ 18 years of age. The lower intradermal dose was immunologically non-inferior to the subcutanous regimen and allowed for more doses of the vaccine to be administered during the outbreak. The vaccination strategy for prevention of mpox had evolved through the outbreak to include high risk individuals through post exposure and pre-exposure use. In the USA over 1 million doses of MVA-BN had been administered and data on vaccine effectiveness had shown that for every one infection among people receiving one dose there had been 14 infections among people receiving no doses of vaccine. Additional studies assessing vaccine uptake and efficacy were ongoing.

LC16 smallpox vaccine

Dr Yasuhiko Shinmura from KM Biologics provided an update on LC16, a third-generation smallpox vaccine which was an attenuated freeze-dried replication-competent *Vaccinia virus* vaccine licensed in Japan in 1975. It was kept in reserves in Japan for emergency use in the case of a smallpox outbreak; in 2013 the WHO recommended 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 since non-clinical pharmacology studies in non-human primates had demonstrated it prevented MPXV infection[35] and there were similarities in immunity to VARV and MPXV. The approved shelf-life at -20 °C had been updated from 4 to 10 years. Vaccine efficacy trials against mpox were needed and being planned.

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. Clinical development of the freeze-dried formulation of MVA-BN had been completed in 2021. Ongoing studies in the Democratic Republic of the Congo had shown an excellent safety profile. Bavarian Nordic was continuing the development of

35 Gordon and al; Smallpox vaccine safety is dependent on T cells and not B cells; J Infect Dis 2011 Apr 15;203(8):1043-53. doi: 10.1093/infdis/jiq16

vaccines based on the MVA-BN vector platform; in April 2022, a candidate for prevention of respiratory syncytial virus (RSV) entered a Phase III clinical trial in adults over 60 years of age with an expected completion in December 2024.

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 and the USA. The global mpox outbreak had increased demand for MVA-BN with more than 70 countries having access to doses of vaccine. 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.[36]

In June 2022, WHO provided interim recommendations on the use of ACAM2000, LC16 or MVA-BN for population groups at risk of mpox during the outbreak. These recommendations were endorsed by SAGE in October 2022.[37] This guidance was echoed by national governments while studies were underway. WHO also called for studies to be carried out on the use of vaccines during the global mpox emergency.[38, 39]

Preliminary information from several studies initiated during the global mpox outbreak suggested that vaccination with MVA-BN was protective against mpox. Studies to further assess effectiveness, immunogenicity and safety of the vaccine were ongoing.

ACAM 2000 vaccine: research, licensing, demand and production update

Dr Laura Cochrane provided an update on ACAM2000 which had been licensed in Australia, Singapore and the USA for the prevention of smallpox. The vaccine was acquired by Canada under a special access program. A plan was underway for a long-term contract with the United States government for delivery to the Strategic National Stockpile, and to support supply to other vaccine reserves worldwide. Manufacturing was ongoing to support demand including potential for surge capacity.

Post marketing studies had been completed, including safety surveillance with retrospective enhanced surveillance in military personnel. An additional IND study for ACAM2000 vaccination in previously ACAM2000-vaccinated plasma donors had been completed. A CDC EAP was noted to be available for vaccination of individuals at risk for non-VARV and OPXV infections during an isolated incident, outbreak or large-scale event. Emergent Biosolutions were committed to supporting further clinical study and investigations.

36 Previously, MVA-BN had mainly been procured for national smallpox vaccine reserves; there was no retail business model in place.

37 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

38 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

39 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

An update was also presented on Vaccinia Immune Globulin (containing antibodies from healthy screened donors immunized with vaccinia vaccine) licensed by the US FDA and Health Canada. It was indicated for treatment of complications due to vaccinia vaccination such as eczema vaccinatum, progressive or severe generalized vaccinia, vaccinia infections in persons with skin conditions, and other aberrant infections caused by *Vaccinia virus*. There was a long-term contract with the US government in place for delivery to the Strategic National Stockpile. Manufacturing was ongoing to support demand. The CDC EAP was noted to be available and named patient requests were available for international use.

Potency testing of the 1st generation vaccines from WHO stockpiles

Dr Jorgen de Jonge of the National Institute for Public Health and the Environment (RIVM), a WHO Collaborating Centre for Smallpox Vaccine, provided an update on potency testing of first- and second-generation smallpox vaccines received from WHO. He reminded the Committee of the mandate of RIVM to perform vaccine potency tests and to ensure *training* for laboratory personnel. In 2022, RIVM set up a new laboratory to conduct potency testing. Between October and November 2022, titration of vaccines was done on batches from the WHO reserves. The potency of four of the five batches tested since 2003 remained stable and in one batch the titre had slightly declined. Overall, the vaccines remained stable, complying with a potency specification of a titer above 10^8 pock-forming units per millilitre. Plans were underway to establish a new dedicated BSL-2 cabinet and increase the number of trained and vaccinated technicians.

Regulatory status of smallpox and mpox vaccine

Dr Andrea Hulse presented an update on licensing and emergency use authorization of *Vaccinia virus* vaccines in the USA for prevention of smallpox and mpox on behalf of the US FDA, based on CDC recommendations and the US FDA regulatory response to mpox in 2021 and 2022. ACAM2000 was recommended for pre-exposure prophylaxis in persons at risk for occupational exposure to orthopoxviruses in June 2015, and in November 2021 this recommendation was expanded to include MVA-BN vaccine. In July 2021, after mpox was reported in the USA,[40] the CDC sponsored an EAP to allow individuals with intermediate- to high-risk exposure to mpox to receive ACAM2000 as post-exposure prophylaxis. No individuals were vaccinated with ACAM2000 and no secondary cases of mpox were reported following those two cases in 2021. In August 2022, mpox was declared a public health emergency in the USA and on 12 August 2022, MVA-BN was further authorized for emergency use at two 0.1 mL intradermal doses, four weeks apart in persons > 18 years of age, and two 0.5 mL subcutaneous doses 4 weeks apart in persons < 18 years of age at high risk.

Dr Lorna Leal Alexander presented an update on the regulatory status of smallpox and mpox vaccines on behalf of the EMA. On 31 July 2013, MVA-BN was approved for immunization against smallpox for persons > 18 years of age. The indication for MVA-BN was expanded on 27 July 2022 to include prevention of mpox. To minimize vaccine shortages in the context of the multi-country outbreak, on 19 August 2022 recommendations were made for intradermal delivery of a fractional dose. There are several ongoing initiatives to better understand safety and effectiveness of MVA-BN against mpox.

40 WHO Disease Outbreak News, Monkeypox – United Sates of America. Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2021-DON344

Discussion on research projects related to vaccines

A number of observations and recommendations were made during the discussion.

The Committee noted that studies on LC16, MVA-BN and VACΔ6 vaccines continued to make good progress and commended VECTOR on the licensure of VACΔ6 in the Russian Federation in 2022. The vaccine had been licensed in persons aged 18 –60 years and there was discussion about the importance to conduct additional studies in immune compromised and pregnant individuals as well as older and younger persons. Colleagues at VECTOR indicated these studies would be forthcoming as would additional studies looking at vaccine stability and shelf life.

Committee Members also highlighted the importance and benefit of vaccine studies undertaken in the Democratic Republic of the Congo. They noted that these collaborations and research projects had begun seven years prior to the global mpox outbreak and the findings of these studies would be important to understand the duration of immunity with pre-exposure vaccination against mpox in this setting. At present, it was noted that antibody response in study participants remained high until day 42 in the MVA-BN studies in the DRC. Meanwhile, antibody response to VACΔ6 (OrthopoxVac) in study participants in the Russian Federation remained high six months after vaccination.

Regarding LC16, MVA-BN and ACAM2000 there were questions regarding manufacturing capacity for which data were noted by suppliers to be confidential. Due to the global mpox outbreak there had been a rise in demand as many governments had continued to procure vaccines. It was noted that the surge capacity for emergency use of MVA-BN had relied on contracts with a small number of national governments. Reserves could not be easily accessed for mpox as they had been set aside for smallpox. Countries had small quantities or none at all of MVA-BN, the vaccine preferred in many countries for reasons of safety in different population groups. Countries with existing stock (Canada, United Kingdom) were able to respond quickly. Regulatory issues and lack of efficacy data for mpox hampered timeliness of response as policy options for deployment versus research were debated. In the event of a smallpox emergency, large quantities of vaccines may be needed at very short notice. It was therefore considered pertinent to examine the possible utility of mRNA multi-antigen vaccines for OPXVs to provide future surge capacity and that use of live variola virus may yet be needed for future vaccine development such as testing of new platform vaccines with respect to specific epitopes.

The work on potency testing of WHO vaccine reserves was well noted and it was suggested to further explore potency testing protocols for all smallpox vaccines. Nonetheless, the Committee recommended that efforts should be made to improve and extend the shelf life of vaccines. Other recommendations on this topic can be found at the end of the report.

Diagnostics

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

Dr Alexander Agafonov 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 generic OPXV immunoassay developed by VECTOR had been validated with *Vaccinia virus*, *Ectromelia virus*, *Rabbitpox virus*, *Cowpox virus* and Monkeypox virus.

Vaccinia virus, ectromelia, cowpox and other viruses such as rubella and measles were used as heterogeneous controls to test the kit. In 2021, using the prototype kit with study samples, a range of orthopoxvirus species (*Vaccinia virus*, *Cowpox virus*, *Rabbitpox virus* and *Ectromelia virus*) in unpurified cell culture preparations with a concentration of 10^3–10^4 PFU/mL had been detected. The assay was specific and easy to use; the kit had five arrays, allowing for samples from five individuals with a wait time of 36 minutes to determine the result. The kit 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. The diagnostic kit had been developed for use by trained health workers and proper methods for disposal of medical waste in the field would be important. The plan for ongoing study was to test the Vector-MPCRrt-Smallpox kit using specimens containing DNA from variola viruses.

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 reported that CDC was continuing collaboration with the ministry of health in the Democratic Republic of the Congo, the Kinshasa School of Public Health and the Institut National de Recherche Bio-Médicale (INRB) in Kinshasa to evaluate nucleic acid-based and protein-based rapid diagnostic tests. Progress had been made in adapting automated diagnostic nucleic acid amplification assays for field settings,[41] and use of this diagnostic platform appeared to be feasible, as heat inactivation of the swab prior to processing would not impair detection.

In the USA, a non-variola virus OPXV PCR assay had been cleared by the FDA, and the generic OPXV PCR test had been submitted for FDA clearance and was made available for use by the national Laboratory Response Network during the mpox outbreak.

Diagnostic development of nucleic acid amplification assays had continued, to validate new reagents and/or equipment (primer/probe chemistries, master mix reagents and extraction technologies) for platforms in use in State and local health laboratories. In 2022, more PCR extraction buffers[42] were identified and optimized for inactivation of MPXV in cell culture and tissue homogenate. Some of the data for these buffers had been submitted to the US FDA for approval for use in the United States Laboratory Response Network.

41 CDC was evaluating the use of the GeneXpert® System for its real time PCR-based assays.
42 Roche® Lysis/Binding buffer and Qiagen® ML buffer.

Dr Hutson noted that regarding protein-based tests, evaluation of a commercial lateral flow assay (Tetracore®) for mpox in the Democratic Republic of the Congo had enrolled just 36 participants. It was found that confirmation of test results in a field setting still required 4.5 days on average due to shipment times and that sensitivity of the assay compared to PCR was low (33%).

In collaboration with Arizona State University, CDC had developed a rapid loop-mediated isothermal amplification (LAMP) test. Evaluation of a LAMP based orthopoxviruses generic assay had promising preliminary results. If successful, CDC would add a variola virus specific target as part of this multiplex assay design.

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. There was discussion about whether the use of NAAT versus protein-based diagnostics would be more useful in the event of a smallpox outbreak. It was felt that while both would be important, protein-based diagnostics would be helpful to quickly assess most situations. Additionally, nucleic acid diagnostic assays must be validated against new sequences and transitioned to newer platforms/reagents. Whereas protein-based and LAMP diagnostic assays would provide valuable flexibility for point-of-care and potential field deployment, there was a need to increase sensitivity. Further development of lateral flow assays which would be useful in a wide range of settings should be encouraged.

The Committee noted that resources developed for smallpox preparedness were now being leveraged for the global mpox outbreak response. The need to find alternative options for diagnostic specimen sampling was noted, such as further exploration of saliva, throat or nasopharyngeal swabs and improved sample processing to enhance virus yield.

Laboratory diagnostic roadmap for mpox

Diagnostic testing for mpox, global laboratory capacity, and WHO response

Dr Lisa Carter provided an update on diagnostic testing and global laboratory capacity for mpox. She reviewed recommendations pertaining to diagnostics from the twenty-third meeting of the Committee. They were to:

1. establish a road map for the development of mpox point-of-care diagnostics;
2. involve regulatory authorities in the development of target product profiles;
3. continue development of OPXV diagnostics with a focus on approaches not requiring the use of live variola virus; and
4. expedite the availability of OPXV diagnostic tests in a reliable and equitable manner.

A survey had been conducted in 2021- 2022, prior to the multi-country mpox outbreak, to map OPXV diagnostic capacity, which assisted the laboratory network during the response. For the mpox response, WHO had supported Member States through publication of interim guidance on laboratory testing for the Monkeypox virus,[43] support on biosafety issues, and training in diagnostics. WHO had supported access to diagnostics by mobilizing expert networks, sharing protocols with regions and countries, and putting in place an international referral mechanism to ship clinical specimens for confirmatory testing. With WHO having also procured near 90 000 commercial molecular tests for over 60 countries across all regions. Early in the outbreak, and despite challenges in scaling up testing capacity due to lack of commercial test kits, in-house assays, and regulatory approvals, most Member States now had access to testing. MPXV diagnostics for animals remained a challenge as PCR was usually negative and serological tests were not well developed.

In addition to these actions to support Member States, WHO was also monitoring MPXV viral evolution, supporting the assessment of commercially available diagnostic kits, planning a global external quality assurance (EQA) programme for MPXV testing and developing target product profiles (TPPs) for diagnostics. Some of these initiatives were further outlined.

Mpox diagnostics Target Product Profile

Dr Jilian Sacks provided the Committee an update on development of diagnostics for MPXV. The global mpox outbreak had increased demand for diagnostics, and the need for simplified assays to be identified was further highlighted, including to facilitate testing at decentralized sites. Evaluation of eleven commercially available PCR kits for detection of MPXV DNA was ongoing.

In 2022, WHO drafted Target Product Profiles (TPPs) for *in vitro* diagnosis of mpox and initiated an expert consultation for their review. Two TPPs were being drafted, the first for detecting MPXV DNA and the other for detecting OPXV viral antigens. Drafts would be made available for public consultation and published with a validity of up to 5 years.

43 WHO. Laboratory testing for the *Monkeypox virus*. Interim Guidance. Geneva, 2022. Available at: https://www.who.int/publications/i/item/WHO-MPX-laboratory-2022.1. Cited 12 April 2023

Evaluation of eleven commercially available PCR kits for the detection of mpox virus DNA

Dr Andreas Nitsche provided an update on an evaluation of 11 recently developed commercial PCR kits intended for research use which required assessment of their clinical performance. The kits showed comparable and high sensitivity to detect Clade I and Clade II MPXV DNA. Limited specificity assessment showed that most assays were specific for MPXV or OPXV.

In discussion, the Committee recommended that the WHO collaborating centres continue to work towards development of (rapid, point-of-care) OPXV diagnostic tests and expedite their availability in a reliable and equitable manner. The Committee recommended that WHO approve continuation through 2023 of previously approved diagnostics development research projects at CDC and VECTOR; and consider development of TPPs for smallpox diagnostics.

Other recommendations on this topic can be found at the end of the report.

Lessons learned from the global mpox outbreak

The Committee undertook to discuss the implications of the 2022 global mpox outbreak, declared on 23 July to be a Public Health Emergency of International Concern (PHEIC), on preparedness for any future smallpox-like event. The discussion covered a range of topics including the development and equitable deployment of medical countermeasures such as vaccines, therapeutics and diagnostics.

The Committee first noted the *ad hoc* meeting convened by WHO in August 2022 to discuss characteristics of MPXV clades and propose names for them. Consensus was reached for nomenclature using a Roman numeral for each clade with lower-case Latin characters for sub-clades; the Congo Basin clade became Clade I and the West African clade Clade II, encompassing two phylogenetically distinct subclades IIa and Ib. While this occurred, parallel discussions took place to consider changing the disease name in the WHO International Classification of Diseases (ICD). WHO issued a public call for new name suggestions for monkeypox in August 2022, and after reviewing over 200 submissions and discussions with stakeholders, it was recommended to use mpox as an inclusion name for monkeypox to be phased in, initially as a synonym, and to become the ICD 'preferred term' after one year.

During the global mpox response, it was observed that some countries were not sharing genome sequences on publicly available databases, although WHO guiding principles and recommendations on the sharing of pathogen genetic sequence data (GSD) were published in 2022.[44] For rapid information-sharing to become standard practice, a globally coordinated laboratory network for genomic sequencing would be required. This would further benefit development of vaccines against emerging infections caused by orthopoxviruses.

The Committee discussed how the global mpox outbreak highlighted how quickly demand for vaccines had risen and the rapidity with which diagnostics could be developed and deployed when political will was present. Members also commented that work done by the collaborating centres has led to promising developments for point-of-care diagnostic tests for smallpox.

Noting the evolution of resistance to tecovirimat in certain patients with mpox and the difficulties providing brincidofovir, it was recommended antivirals be further developed. It was also noted that while mAbs had a place for treatment, they would not be widely available and would be expensive so development and discovery of small molecule antivirals should be prioritized for use by themselves or in combination to give maximal protection in the event of a smallpox event and to delay or slow down the emergence of resistance.

Both COVID-19 and mpox had raised awareness among the general population about public health measures and the use of diagnostic tests, vaccines and new technologies against emerging biological threats. Both outbreaks demonstrated the importance of global supply chain coordination when there is global demand for resources.

44 World Health Organization. (2022). WHO guiding principles for pathogen genome data sharing. World Health Organization. https://apps.who.int/iris/handle/10665/364222. Cited 15 April 2023.

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 2020–2022 outlined at the twenty-third meeting of the Committee was presented in the report of that meeting. In 2021, and not withstanding delays related to the mpox outbreak and COVID-19 pandemic, there were no major departures from the agreed roadmap.

Since the research roadmap initially outlined was for three years to 2022, the Committee agreed by consensus to extend the time frame by one year for previously approved projects which had not been completed, to include 2023. Minor adjustments are highlighted in Table 1, offering the reader continuing visibility on the work expected to be completed ahead of the next discussion by Member States at the World Health Assembly in 2024. Detailed proposals will continue to be reviewed annually. The recommendations of the Committee at the twenty-fourth meeting are summarized in the section that follows Table 1.

Table 1. Programme of research using live variola virus presented by WHO collaborating centre repositories for 2020 to 2023 (with updates)*

Area of work	CDC	VECTOR
Genome sequencing	Complete genome sequencing of 40 isolates. *Updated to include isolates sampled from the long-term repository vault in 2022.*	Complete the genome sequencing of 50 of the remaining 88 isolates (2023).
Diagnostics	Adapt and optimize multiplex nucleic acid tests for new platforms and field settings. Continue development and optimization of protein-based tests.	Optimize the design of the immunochemistry test kit and its accessories using OPXV, including Variola and Monkeypox viruses.
Antivirals	**Tecovirimat** 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** Continue to study in vitro activity (EC_{50}) of antiviral candidate ST-357 and optimized analogues to select preclinical candidates against variola virus. **Monoclonal antibodies and antibody mixes** 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).*	**NIOCH-14 oral formulation** Assessing 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. **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.* **Monoclonal antibodies and antibody mixes** Evaluate antivirals against -variola virus based on monoclonal antibodies.
Vaccines	**MVA-BN and LC16m8** Finalize efficacy testing on long-term titre samples from MVA-BN and/or LC16m8 vaccine trials (as samples are available).	**VACΔ6** 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.
Animal models	**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.	

* Edits in italics are new from November 2022 compared to the roadmap agreed in 2021. Note: This multi-year research agenda was presented to the WHO Advisory Committee on variola virus Research at their 21st, 22nd, 23rd and 24th meetings, derived from collaborating centre proposals and presentations.

Recommendations

Committee members and the Secretariat discussed the research proposals shared during the meeting. Among the recommendations shared below, the Committee noted several points with respect to antivirals, vaccines and diagnostics being developed for orthopoxviruses and lessons learned from the multi-country mpox outbreak.

The Committee pointed out that development and licensure of three antiviral drugs (tecovirimat, brincidofovir and NIOCH-14) was an important accomplishment, based on the mandate accorded at the Seventy-second World Health Assembly. Despite the success of antiviral drug development, members expressed concern that early signals of viral resistance to tecovirimat had been detected in two patients during the multi-country mpox outbreak and what that could mean for a larger scale and more virulent smallpox outbreak. Committee members felt that further investment in development of smallpox antivirals and therapeutics with different mechanisms of action was justified and that ultimately combination therapy would be best. For mpox, antiviral studies had not to date noted any safety signals and should continue to better understand and document efficacy.

The Committee also noted the promising work related to third- and fourth-generation vaccines. Concern was raised over the limited production capacity for licenced OPXV vaccines. The Committee also noted that licensed GMP-compliant third and fourth generation vaccines should be considered for inclusion in national and WHO reserves.

Regarding diagnostics, the Committee noted the work done by the WHO during the global mpox response and the development of two Target Product Profiles. The Committee noted that as MPXV is often used as a study surrogate for variola virus, resources developed for smallpox preparedness will continue to be vital to control a continued or accelerated emergence of mpox, as that posed the most significant public health threat from OPXVs, and that this was a major immediate benefit of the research undertaken under the oversight of WHO.

The recommendations of the Committee as articulated at its twenty-fourth meeting are summarized below. Text in parentheses indicates to whom each recommendation is directed.

General

- Extend the current three-year research roadmap to four years, to 2023 (WHO).
- Approve continuation through 2023 of previously approved research projects at CDC and VECTOR; approve amendment requested by CDC for new collaboration on mAb development; and request a written update in mid-2023 from WHO collaborating centres in advance of the twenty-fifth ACCVR meeting (WHO).
- Complete full genome sequencing of variola virus strains or isolates without amplification of virus, and make sequence data for all available isolates publicly available directly or via WHO as soon as possible (WHO collaborating centres).
- Continue development projects for smallpox antivirals, vaccines and diagnostics as approved by WHO in 2019, 2020 and 2021 but delayed by the COVID-19 and mpox outbreaks (WHO collaborating centres).
- Consider a tabletop exercise for a smallpox event in preparation for logistics, supply chain of products and mitigation strategies to forestall large scale spread (WHO).
- 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 (WHO and WHO collaborating centres).

Antivirals

- Continue development and discovery of small molecule antivirals for use by themselves or in combination to give maximal protection in case of a smallpox event and to delay or slow down the emergence of resistance (WHO and WHO collaborating centres).
- Continue work to identify genetic markers of resistance to antivirals (WHO collaborating centres).

Vaccines

- 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 collaborating centres and WHO).
- Continue work to characterize and harmonize potency testing protocols for all smallpox vaccines (WHO collaborating centres and WHO).
- Continue efforts to improve the shelf life of vaccines (vaccine manufacturers).
- Include minimally- or non-replicating vaccines in WHO strategic reserves (WHO).

Diagnostics

- Continue work on roadmap to leverage advances in smallpox diagnostics for further development of point-of-care diagnostics for mpox (WHO).
- 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).
- 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).
- Consider development of target product profiles for smallpox diagnostics (WHO).

Annexes

Annex 1 | Agenda

Agenda

Objectives of the meeting

- Review progress of approved research with live variola virus;
- Review research programme and recommendations for 2020-2022; and
- Develop research agenda for 2023.

Tuesday 29 November 2022

BRIEFING SESSION FOR NEW MEMBERS		
9h00	WHO Advisory Groups: Overview	**Ms D. Durcheva**, WHO
9h10	Terms of Reference of the Advisory Committee on variola virus Research	WHO Smallpox secretariat
9h20	Brief history of the Advisory Committee on variola virus Research	**Dr D. Ulaeto** Chair
9h30	**Coffee break**	
Session 1. Opening and report of the WHO Smallpox Secretariat		
10h00	Opening remarks	**Dr M. Ryan**, Executive Director, WHO Health Emergencies Programme -
10h20 10h30	Roll call, Declarations of Interest Report of the Smallpox Secretariat	WHO Smallpox Secretariat **Dr R. Lewis**, Head, WHO Smallpox Secretariat
11h00	**Discussion**	*All*
Session 2. WHO COLLABORATING CENTRE REPORTS - variola virus and dna collections		
11h40	Summary of proposals for research with live variola virus	Chair ACVVR
11h50	Report on the variola virus collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for variola virus Strains and DNA *and* Overview of major activities to develop countermeasures against variola virus and other human pathogenic orthopoxviruses (Approved 2020)	**Dr R.A. Maksyutov**, Director General, FBRI SRC VB VECTOR, Rospotrebnadzor, Russian Federation
12h00	Report on the variola virus collection at the WHO Collaborating Centre for Smallpox and Other Poxviruses – Centers for Disease Control, USA *and* Use of live variola virus to maintain and regenerate non-infectious variola virus-derived materials for diagnostic development support (Approved 2020)	**Dr C. Hutson**, Lead, Virus-Host Molecular Interactions Team (VHMI), Centres for Disease Control, Atlanta, Georgia, USA
12h10	**Discussion**	*All*

Session 3. ANTIVIRALS - Progress reports		
12h25	Clinical trials on the anti-smallpox drug NIOCH-14 *and* Discovery and testing novel chemical antivirals for smallpox treatment and prevention (Approved 2019)	**Dr A.P. Agafonov** VECTOR
12h40	**Discussion** on VECTOR antivirals research projects	*All*

13h00	**Lunch Break (1hr)**	
14h00	Use of live variola virus to characterize the effectiveness of	**Dr T. Smith,** CDC
14h10	antiviral therapeutic tecovirimat (additional data, Approved 2019) and - Use of live variola virus to characterize the effectiveness of novel antiviral therapeutic ST-357 (Approved 2019) - Use of live variola virus to determine whether mice are a suitable animal model for human smallpox (Partially approved 2020; Approved 2021)	**Dr T. Smith,** CDC
14h20	Tecovirimat and ST-357 – licensing & production update	**Dr D. Hruby,** SIGA Technologies
14h30	Brincidofovir – licensing & production update	**Dr L. Cochrane,** Emergent Biosolutions
14h40 14h55	Regulatory status of smallpox and monkeypox antivirals **Discussion** on CDC antivirals research projects	**Dr P. Harrington**, US FDA **Dr L. Leal Alexander**, EMA *All*

Session 4. MONOCLONAL ANTIBODIES - Progress reports		
15h15	Evaluation of antivirals against smallpox based on monoclonal antibodies. (Approved 2019) Replenishment of the stocks with non-infectious material, derived from live variola virus required for diagnostics development (Approved 2020)	**Prof S.N. Shchelkunov** VECTOR
15h25	Use of live variola virus to evaluate antivirals (monoclonal biologics) against variola virus (Approved 2019)	**Ms A. Matheny** CDC
15h35	**Discussion** on monoclonal antibody research projects	*All*

16h05	**Close of day one**

Wednesday 30 November 2022

Session 5. VACCINES - Progress reports		
09h00	Recap of discussions and recommendations of Day 1	Chair
09h30	Clinical trials of the VACΩ6 vaccine against smallpox and other orthopoxvirus infections. Assessment of the neutralizing activity of vaccinated volunteers' sera and those of animals following vaccination with the OrthopoxVac and with new vaccine variants using variola virus to support the development of less reactogenic fourth generation smallpox vaccine (Approved 2019)	**Prof S.N. Shchelkunov** VECTOR
09h40	**Discussion** on VECTOR vaccine research	*All*
10h00	**Coffee break**	
10h05	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, the Democratic Republic of the Congo	**Dr T. Smith** CDC
10h15	LC16 smallpox vaccine – research, licensing, demand and production update	**Dr Y. Shinmura** KM Biologics
10h25	MVA-BN vaccinia vaccine – research, licensing, demand and production update	**Dr F. Lienert** Bavarian Nordic
10h45 10h50 11h05 11h20	ACAM 2000 vaccine - research, licensing, demand and production update Potency testing of vaccines from the stockpiles Regulatory status of smallpox /mpox vaccine **Discussion** on CDC vaccine research updates and plans	**Dr L. Cochrane,** Emergent Biosolutions **Dr J. de Jonge**, RIVM **Dr A. Hulse/D. Kaslow**, US FDA **Dr L. Leal Alexander**, EMA *All*
11h50	**LUNCH BEAK (1hr)**	

Session 6. DIAGNOSTICS - Progress reports		
12h50	Development of advanced methods for rapid point-of-care diagnostics of orthopoxvirus infections. (Approved 2020)	**Dr A.P. Agafonov** VECTOR
13h00	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 C. Hutson,** CDC
13h10	**Discussion** of diagnostic research updates, plans, next steps, and future plans for the development of diagnostics without use of variola virus	*All*

Session 7: Laboratory support to global monkeypox response and diagnostic roadmap		
13h30	Diagnostic testing for monkeypox, global laboratory capacity, and WHO response	**Dr L. Carter,** WHO
13h40	Monkeypox Diagnostics Target Product Profile	**Dr J. Sacks,** WHO
13h45	Evaluation of eleven commercially available PCR kits for the detection of Monkeypox virus DNA	**Dr A. Nitsche,** RKI
14h00	Lessons learned from the global mpox response	*All*

14h20	Coffee Break (10 minutes)

Session 8. CLOSED SESSION for ACVVR members and advisers		
14h30	**Discussion** on live variola virus research agenda 2023, recommendations, and pending issues	**Dr D. Ulaeto, Chair**
14h50	**Draft recommendations**	

16h30	**Close of day two and ACVVR24**

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, ICMR-National Institute of Virology, 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, 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 (retired)

Dr Robert Drillien, 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, CA, United States of America

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, Director, National Biodefense Analysis and Countermeasures Center; President, Battelle National Biodefense Institute, Frederick, Maryland, United States of America

Dr Rinat A. Maksyutov, Director General, Federal Budgetary Research Institution State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Koltsovo, Russian Federation

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

Dr Mohamed Moussif, Chief Medical Officer and national coordinator of the 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, Tenure researcher, Israel Institute for Biological Research, Ness Ziona, Israel

Professor Geoffrey L. Smith*, Head, Department of Pathology, University of Cambridge, Cambridge, 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 Delia A Enria (for STAG-IH), Former Director, Instituto Nacional de Enfermedades, Virales Humanas, Buenos Aires, Argentina

Dr David Kaslow, Office Director, Office of Vaccines Research & Review, Center for Biologics Evaluation & Research, U.S. Food & 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 Masayuki Saijo, Director, Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan

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.

(*) Did not attend.

Invited presenters (WHO collaborating centres)

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

Dr Jorgen de Jonge, Senior Scientist preclinical influenza vaccines, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands (Kingdom of the)

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, United States of America

Ms Audrey Matheny, 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 Brett Petersen, Captain, U.S. Public Health Service, Deputy Chief, 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

Professor Sergei N. Shchelkunov, Chief Scientist, 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 (PRB), Division of High Consequence Pathogens and Pathology (DHCPP), National Center for Emerging and Zoonotic, Infectious Diseases (NCEZID), Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA

Invited presenters (others)

Dr Laura Cochrane, VP, Global Medical Affairs, Medical Countermeasures, Emergent Biosolutions, Rockville, Maryland, USA

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, 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

Dr Dennis E. Hruby, Chief Scientific Officer, SIGA Technologies Inc., Corvallis, Oregon, United States of America

(*) Did not attend.

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

Dr Florian Lienert, Product Lead Medical Affairs, Bavarian Nordic GmbH, Martinsried, Germany

Mr Yasuhiko Shinmura, Manager, Development Department, R&D Division, Kikuchi Research Center, KM Biologics, Kumamoto, Japan

World Health Organization

Regional offices

Representative for the WHO Regional Office for Africa*

Representative for WHO Regional Office for the Americas* (Dr Jairo Mendez Rico, Advisor in Viral Diseases)

Representative for the WHO Regional Office for the Eastern Mediterranean* (Dr Amgad Elkholy, Medical Officer)

Representative for the WHO Regional Office for Europe*

Representative for the WHO Regional Office for South-East Asia (Dr Manish Kakkar, Technical Officer, High Threat Pathogens)

Representative for the WHO Regional Office for the Western Pacific (Dr Sangjun Moon, Medical Officer, HIM)

Headquarters

Dr Sylvie Briand, Director, WPE/EPP

Dr Lisa Carter, Technical Officer, WPE/CRS/PHL

Ms Alexandra Hill, Technical Officer, WPE/EPP/IEP

Dr Kazunobu Kojima, Technical Officer, WPE/EPP/BSP

Dr Rosamund Lewis, Head, Smallpox Secretariat, WPE/EPP/EZD

Dr Catherine Makison Booth, Technical Officer, WPE/EPP/BSP

Dr Bernard Onoja, Technical Officer, WPE/EPP/EZD

Dr Isis Pluut, Scientist, MHP/RPQ/PQT/VAX

(*) Did not attend.

Dr Mike Ryan, Executive Director, WHE

Dr Jilian Sacks, Technical Officer, WPE

Ms Josine Umugwaneza, Assistant to Team, WPE/EPP/EZD

Dr Maria Van Kerkhove, Unit Head, WPE/EPP/EZD

Annex 3 | Research proposals

Annex 3a. Research proposals presented for 2023 by VECTOR, and WHO approval status

Proponent and projects	Yes	No	Majority opinion and notes	Approval date
VECTOR **Use of live variola virus to:**	**Recommendation of ACVVR members who reviewed proposals**		**Recommendation of 23rd ACVVR**	
1. Assess the neutralizing activity of vaccinated volunteers' sera and the sera of vaccinated animals to support the development of less reactogenic fourth generation smallpox vaccines [continuing]			Continuation* Amendment request pending with VECTOR for work with new vaccine strains (not approved by WHO in this round)	November 2019
2. Discover and test novel chemical antivirals for smallpox treatment and prevention [continuing]			Continuation*	November 2019
3. Evaluate antivirals against smallpox based on monoclonal antibodies [continuing]			Continuation*	November 2019
4. Replenish the stocks with non-infectious material, derived from live virus, required for diagnostics development [recurrent]			Continuation*	November 2020
5. Develop advanced methods for rapid (point-of-care) diagnostics of orthopoxvirus infections [continuing]			Continuation*	November 2020

* Delays occurred in 2020 - 2022 due to the COVID-19 pandemic and building renovations.
There was consensus in the Committee to endorse continuation of previously approved projects.

Annex 3b. Research proposals presented for 2023 by CDC, and WHO approval status

Proponent and projects	Yes	No	Majority opinion and notes		Approval date
CDC **Use of live variola virus to:**	**Recommendation of ACVVR members who reviewed proposals**			**Recommendation of 23rd ACVVR**	
1. Maintain and regenerate non-infectious variola virus derived materials for diagnostic development support [recurrent]				Continuation*	November 2020
2. Characterize effectiveness of antiviral therapeutic [tecovirimat] [completing]				Continuation*	December 2019
3. Characterize effectiveness of novel antiviral therapeutic ST-357 [continuing]				Continuation*	December 2019
4. Evaluate antivirals (monoclonal biologics) against variola virus [+amendment] [continuing]	15	0	Yes	Continuation* Amendment requested at the 24th ACVVR meeting; approved in 2022 by WHO after consultation of Committee members	December 2019 Amendment: December 2022
5. Determine whether mice are a suitable animal model for human smallpox, providing means to evaluate medical countermeasures against authentic agent [+amendment] [continuing]				Continuation*	November 2020 Amendment: December 2021
6. Support less reactogenic vaccine development: continued evaluation of third generation vaccines [continuing]				Continuation*	December 2019
7. Develop protein-based diagnostic and detection assay specific for variola virus [continuing]				Continuation*	December 2019

* Delays occurred in 2020 and 2021 due to the COVID-19 pandemic and global mpox outbreak response. There was consensus in the Committee to endorse continuation of previously approved projects.

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

Shchelkunov S.N., Agafonov A.P., Maksyutov R.A.
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 with WHO recommendations. Based on these, working instructions (SOPs) 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 Africa, Asia, Europe, the Middle East and South America, maintained in a dedicated repository in freeze-dried or frozen form.

In 2022, research was conducted using live variola virus to evaluate the neutralizing activity of sera from vaccinated human volunteers as part of the clinical trial of the VACΔ6 vaccine.

The work with live variola virus is scheduled to continue in 2023 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 volunteers' sera and those of animals following vaccination with the OrtopoxVac vaccine and with new vaccine variants, using variola virus to support the development of less reactogenic fourth-generation smallpox vaccines.
3. Discovery and testing of novel chemical antivirals for smallpox treatment and prevention.
4. Use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies.
5. Development of advanced methods for rapid (point-of-care) diagnostics of orthopoxvirus infections.

Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development

Ovchinnikova A.S., Kabanov A.S., Odnoshevskiy D.A., Tregubchak T.V., Shchelkunov S.N., Maksyutov R.A.
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 are determined by the need to develop sensitive and highly specific diagnostic tools, and they remain essential up to the present.

Since 2001, FBRI SRC VECTOR, Rospotrebnadzor, has been carrying out work on the isolation of variola virus (VARV) genomic DNA and its preservation in the collection of DNAs of VARV strains, which currently includes 39 DNA preparations of various VARV strains.

In 2018, the work was performed to isolate DNA from the following five variola virus strains: Nep-2, Botsw-92, Eth-187, India 378, and Aslam.

To avoid additional passages of the virus, VARV DNA was isolated from the materials of the early passages of strains India 378 and Aslam maintained in the collection as well as from scabs collected from human skin that contained strains Nep-2, Botsw-92, Eth-187, which viral DNA had not previously been isolated.

As a result of whole genome sequencing, the presence of VARV DNA in the studied samples from VECTOR's collection was confirmed. However, the amount of initial material did not allow identifying complete nucleotide sequences of the VARV genomes of the strains Nep-2, Botsw-92, and Eth-187

In 2020, we modified the technique for isolating orthopoxvirus DNA. This technique was tested with preparations containing *Ectromelia virus*, *Vaccinia virus*, and *Cowpox virus*, and it showed high efficiency, i.e. the DNA sequences of these viruses were successfully determined.

In 2022, Monkeypox virus DNA was successfully isolated from human clinical specimens using a modified technique, and the complete nucleotide sequence was also determine.d

In 2023, our task is to isolate DNA of VARV 50 strains, which will be stored in the collection of FBRI SRC VB VECTOR, Rospotrebnadzor, and to conduct whole genome sequencing of these isolated VARV DNAs. The established DNAs of VARV strains will be used to evaluate the effectiveness of the medical device: the reagent kit "Vector-MPCRrt-Smallpox" (Marketing Authorization No. RZN 2016/3685).

Discovery and testing of novel chemical antivirals for smallpox treatment and prevention

Agafonov A.P., Shishkina L.N., Bormotov N.I., Serova O.A., Kabanov A.S., Ovchinnikova A.S., Odnoshevskiy D.A., Yurganova I.A., Pyankov O.V., Maksyutov R .A.
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

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

In 2019-2022, 505 new chemical compounds of different classes were tested in surrogate orthopoxviruses (vaccinia, cowpox, and *Ectromelia* viruses) *in vitro*. Among them, there were compounds containing a monoterpene scaffold or fragments of camphor, borneol and adamantane 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 hydroxyimidazole derivatives containing ester or amide fragments synthesized at D.I. Mendeleev University of Chemical Technology of Russia (D.I. Mendeleev RCTU). Cidofovir was used as a reference drug.

Among the tested compounds, 25 compounds were identified whose selectivity indices (SI) against the surrogate orthopoxviruses mentioned above ranged from 100 to 18 100. The most active compounds against surrogate orthopoxviruses were chemical structures containing fragments of monoterpenes, camphor, borneol, adamantane, isobornylamine, phenylamides, benzylamides, bicyclic backbone, and 2-aryl-1-hydroxyimidazole derivatives. These compounds are scheduled to be enrolled for testing of their antiviral activity against variola virus (VARV) in Vero cell culture in 2023.

In addition, in 2022, the antiviral efficacy of NIOCH-14 was evaluated in Vero E6 cell culture against a new strain of Monkeypox virus (MPXV), St.Petersburg-22, isolated from clinical material of a person with monkeypox in July 2022. It was found that the 50% virus inhibitory concentration (IC50) of NIOCH-14 for the MPXV St.Petersburg-22 strain was 0.02 μg/ml, while SI was >15 000.

In our previous experiments, it had been demonstrated that IC50 of NIOCH-14 against the MPXV V79-1-005 strain was 0.013 μg/ml, and SI was >7 700. This proves that NIOCH-14 exhibits high *in vitro* antiviral activity both against MPXV variants previously circulating in the world and against the new MPXV strain isolated in 2022.

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 variola virus.

In October 2022, NIOCH-14 was licensed in the Russian Federation for treatment of smallpox, monkeypox and cowpox.

Evaluation of antivirals against smallpox based on monoclonal antibodies

Merkuleva Yu.A., Shanshin D.V., Shchelkunov S.N., Shcherbakov D.N.
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, Russin Federation

The FBRI - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (Russian Federation) has developed preparations of monoclonal antibodies, A31, B23, H72, and M12B9ch, specific to the immunodominant orthopoxvirus proteins, A27, B5, H3 and L1. All the antibodies were generated in the scFv-Fc format, with A31, B23, and H72 being human antibodies and M12B9ch being a chimeric monoclonal antibody containing the VH/VL domains of a mouse antibody. For the production of antibodies, stable producers were generated based on the CHO-K1 cell line of Chinese hamster ovary. The conditions were optimized to culture producers and purify the recombinant antibodies from the culture medium using the A protein affinity chromatography.

All the antibody preparations specifically interacted in ELISA with a lysate of the LIVP *Vaccinia virus* strain. To assess the specificity of the final preparations, recombinant variants of the variola virus proteins, A30, B7 and M1 (orthologs of A27, B5, and L1), were also developed. The nucleotide sequences encoding the ectodomains of the A30, B7, and M1 proteins were amplified by PCR using a variola virus genome library. To obtain the H3 protein, a DNA fragment encoding the consensus amino acid sequence of the H3 protein of variola, cowpox and mpox viruses was used. The protein sequences were His-tagged and cloned into the pVEAL2 integrative plasmid vector using the Sleeping Beauty transposon system. Then, stable strains of producers of all the proteins were obtained based on the CHO-K1 cell line; the proteins were produced and purified using Ni-affinity chromatography. The purified proteins were analyzed in ELISA using the sera of vaccinated individuals, as well as the sera of rabbits immunized with *Vaccinia virus*.

The monoclonal antibodies, A31, B23, H72, and M12B9ch specifically interacted with the derived recombinant proteins, A30, B7, M1, and H3 in ELISA and dot-blot analysis.

The next step of the work involved the evaluation of the neutralizing activity of the derived monoclonal antibodies against *Vaccinia virus* in Vero cell culture *in vitro*. The plaque reduction neutralization test against the LIVP *Vaccinia virus* strain (PRNT50) showed that all the three human monoclonal antibodies neutralized *Vaccinia virus* at a concentration of 1.5-7.6 μg/ml, and the M12B9ch chimeric antibody - at a concentration of 0.125 μg/ml, in the absence of the complement system components. The M12B9ch antibody in the presence of 2.5% human complement showed neutralizing activity at a concentration of 0.002 μg/ml. It will be proposed that the developed monoclonal antibodies be further tested for their neutralizing activity against variola virus and other orthopoxviruses.

Assessment of the neutralizing activity of volunteers' sera and those of animals following vaccination with the OrtopoxVac vaccine and with new vaccine variants, using variola virus to support the development of less reactogenic fourth-generation smallpox vaccines

Shchelkunov S.N., Ovchinnikova A.S., Kabanov A.S., Odnoshevskiy D.A., Yurganova I.A., Gavrilova E.V., Maksyutov R.A.
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 OrtopoxVac, for prevention of smallpox, monkeypox, and infections due to other orthopoxviruses.

Development of advanced method for rapid (point-of-care) diagnostics of orthopoxvirus infections

Agafonov A.P., Poltavchenko A.G., Ushkalenko Y.D., Filatov P.V., Yorsh A.V., Yakubitskiy S.N., Sergeev A.A., Shchelkunov S.N., Maksyutov R.A.
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 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 Monkeypox virus, *Vaccinia virus*, *Cowpox virus*, *Rabbitpox virus* and *Ectromelia virus* has 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 scabs of the pustules at the vaccination site in patients.

This kit remains stable at 4 to 8 ° C for 18 months. 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 out-of-laboratory settings.

The plan of further work includes the kit's testing using variola virus pathogenic for humans.

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

Christine Hughes, Todd Smith, Audrey Matheny, Kimberly Wilkins, Yu Li, Hui Zhao, and Christina Hutson

WHO Collaborating Center for Smallpox and other Poxvirus Infections, Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

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. Most of these viruses were originally isolated on embryonated eggs and characterized during the final years of the intensification of the smallpox eradication campaign. The virus collection is maintained in two separate freezers, one of which is a back-up freezer remaining largely untouched.

In the USA, variola virus is a select agent and is subject to the select agent regulations (42 CFR part 73). Secure databases addressing WHO recommendations and USA 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 2020 and 2022.

Since the 23rd ACVVR meeting, WHO-approved research activities using 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) use of live variola virus to characterize effectiveness of anti-viral therapeutic (TPOXX). In 2022, sample analysis of original isolates removed in 2020 from the repository freezer was conducted. Last year we sequenced two variola samples: VARV_AFG70_V4_P9 –passaged 9 times in the cell culture and VARV_AFG70_V4_P45 -passaged 45 time in cell culture.

The preliminary analysis shows that both new sequences have one single nucleotide polymorphism (SNP) compared to the published genome sequences of VARV_AFG70_V4, and the sample with 45 passages contains two additional SNPs. Sequencing and analysis are currently underway for 40 isolates removed from a long-term freezer in 2022; 36 of these are believed to be unique VARV isolates.

The laboratory space was in active use from 20 July 2021 to 28 March 2022; the laboratory underwent decontamination prior to preventive maintenance during April 2022 and remained “cold” (inactive) for approximately 2 months.

During May 2022, WHO conducted an inspection; the final inspection report is pending. The laboratory became “hot” (active) again on 25 May 2022. During June 2022, the Federal Select Agent Program conducted an inspection and had one minor finding relating to document review.

Use of live variola virus to characterize effectiveness of antiviral therapeutic (tecovirimat)

Todd Smith, Audrey Matheny, Christine Hughes, Yu Li, and Christina Hutson

WHO Collaborating Center for Smallpox and other Poxvirus Infections, Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

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 US FDA for treatment of smallpox. Tecovirimat has been tested extensively *in vitro* and *in vivo* against multiple orthopoxviruses. It has been the frontline treatment for the 2022 mpox outbreak in the USA. 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 tecovirimat, the F13L protein.

Previously 215 variola virus genomes were analyzed identifying 10 F13L amino acid variants. In 2022, no new genomes were analyzed. A key component of the post-marketing requirement/ commitment was testing the antiviral activity of tecovirimat against an expanded panel of VARV isolates. Previously reported results showed all isolates tested were sensitive to tecovirimat.

In 2022, one isolate from a previously identified amino acid variant was tested for tecovirimat sensitivity using a cytopathic effect (CPE) assay. This isolate was found to be sensitive to tecovirimat with a half-maximal effective concentration (EC_{50}) of 0.018 µM, and a 90% effective concentration (EC_{90}) of 0.048 µM. This result is consistent with previously reported results showing seven variola virus isolates from five amino acid variants were sensitive to tecovirimat with EC_{50} range of 0.01–0.03 µM and EC_{90} range of 0.02–0.15 µM. For the remaining isolates that are unavailable for tecovirimat sensitivity testing and to avoid creating a recombinant virus that contains VARV sequences, a strategy was adopted of expressing the unavailable F13L amino acid variants in stable cell lines. Tecovirimat sensitivity will be evaluated using a CPE assay by infecting the stable cell lines with a *Vaccinia virus* IHD-J with F13L deleted (approved by CDC Institutional Biosafety Committee: 2019.394). Currently, generation of the stable cell lines is ongoing.

Continuing research proposals for 2023 were presented at the twenty-fourth meeting of the WHO Advisory Committee Variola Virus Research which took place in November 2022.

Use of live variola virus to characterize effectiveness of novel antiviral therapeutic ST-357

Todd Smith, Audrey Matheny, Christine Hughes, Yu Li, and Christina Hutson

WHO Collaborating Center for Smallpox and other Poxvirus Infections Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

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

Development of antiviral treatment strategies is a component of smallpox bioterrorism preparedness. Additional medical countermeasures would ensure options exist should one treatment fail. An 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 that of tecovirimat and brincidofovir.

During the first year of proposal approval (2019–2020), the parental compound ST-357 was screened against three isolates of live variola virus, and two ST-357 analogues were screened against *Vaccinia virus* strain Western Reserve. All three variola virus 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*. Since 2020, no work with live variola virus has been done under this project while additional ST-357 analogues with improved solubility characteristics are sought.

Evaluation of monoclonal biologics against live variola virus

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

The WHO Collaborating Center for Smallpox and other Poxvirus Infections, Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

Additional external collaborators: *Iuliia Gilchuk, James Crowe, Jr., (the Vanderbilt Vaccine Center); Darryl Sampey (BioFactura)*, and Biomedical Advanced Research and Development Authority

The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox re-emerges. In the USA, variola virus (VARV) is subject to Select Agent Regulations (42 C.F.R. part 73). Therapeutic strategies are important for outbreak response efforts as well as disease treatment and ideally would have discrete mechanisms of action to be licensed and available for use. Single amino acid changes in orthopoxviruses can render resistance to TPOXX® (tecovirimat) treatment, and there are safety concerns for treatment with Tembexa® (brincidofovir) for certain individuals. In this context, it could be beneficial to have a monoclonal antibody (mAb) cocktail for orthopoxviruses with at least two mAbs to target both infectious forms of the virus (extracellular enveloped virions (EV) and intracellular mature virions (IMV)).

After several years of testing mAbs developed by Vanderbilt University in plaque reduction neutralization (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 plans to evaluate the mixes against MPXV using the Cast/EiJ mice, and to continue to screen mixes against VARV and MPXV with PRNT.

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 Biomedical Advanced Research and Development Authority (BARDA) awarded an Advanced Research and Development Contract 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 and MPXV for humanized variants (current production runs from non-clonal stable pools) for two potential mAb drug product components [anti-VACV L1 (7D11), and anti-VACV B5 (8A)]. The anti-L1 humanized antibody (h7D11) neutralized the IMV form of VARV by PRNT with EC50 concentrations of <0.01 µg/mL, with or without complement.

These results were consistent with previous EC50 concentrations using earlier productions runs of this antibody. The anti B5 humanized antibody (h8A) neutralized the EV form of VARV by PRNT with EC50 concentrations around 0.2 µg/mL. This value was approximately 58% higher than previously reported values for earlier production runs of that antibody. The h7D11 and h8A mAbs were also screened against the IMV and EV forms of MPXV by PRNT. The EC50 concentration of h7D11 ranged from 0.005 – 0.02 µg/mL in the presence/absence of 10% complement against the IMV form, while the EC50 concentration of h8A was 0.4 µg/mL against the EV form. It was concluded that larger scale production should continue for both h7D11 and h8A mAbs with *in vitro* neutralization against VARV and MPXV continuing at critical production steps.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-fourth ACVVR meeting which took place in November 2022.

Use of live variola virus to determine whether humanized mice are a suitable animal model for human smallpox

Investigators: Todd Smith, Cassandra Tansey, Jana Ritter, Theodora Khan, Audrey Matheny, and Christina Hutson
The WHO Collaborating Center for Smallpox and other Poxvirus Infections, Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

To date, non-human primates (NHPs) are the only non-human animal species which exhibit disease with variola virus (VARV) infection. Limitations with the NHP model and other surrogate orthopoxvirus models, such as short disease incubation periods which do not resemble human smallpox, make these systems suboptimal for evaluating efficacy of therapeutics after symptom onset. We have shown that humanized mice are a more permissive/representative VARV animal model to facilitate better testing of therapeutics. We previously published detailed results showing three types of humanized, female 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 utilized due to late disease progression and early development of graft-versus-host disease. Both the hu-CD34 and BLT mouse models showed systemic infection around day 9–10 post infection, which is approximately 3–4 days before mortality, allowing for therapeutic efficacy testing in these models.

Demonstrating efficacy against live VARV *in vivo* may be required should another anti-VARV therapeutic attempt to gain licensure. Our current goal is to validate both the hu-CD34 and BLT models by demonstrating the efficacy of TPOXX (tecovirimat) in these models. We have completed the validation in hu-CD34 mice treated with tecovirimat 0, 1 or 2 days post-VARV challenge. Results showed statistically significant protection for all tecovirimat 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 are prepared to complete the same validation study with the BLT mouse model in January 2023. By characterizing this unique animal model, the global community will have vital information concerning its utility for testing future therapeutics against VARV *in vivo*.

Part of this project is to identify early biomarker(s) of smallpox disease. Biomarker molecular assays continued in 2022, using both MAGPIX and NanoString® technologies for the hu-CD34 and BLT models. Magpix utilizes the principles of flow cytometry and enables simultaneous measurement of up to 50 analytes. Nanostring is an amplification-free technology that measures host nucleic acid expression. Preliminary results showed a large down regulation of B-cell markers in the spleen. CCL7, a monocyte attractant gene, is the only gene with significant and relevant activation that is sustained across the time course. Further characterization of these samples could be useful for identifying potential early/pre-symptomatic biomarkers of smallpox infection (similar to that seen with both human Ebola samples and NHP Ebola challenge studies). Detecting infected but asymptomatic patients could allow focused use of medical countermeasures and non-pharmaceutical interventions to minimize spread of disease, which the ongoing COVID-19 pandemic has reinforced as crucial for protecting lives.

Progress under this proposal and plans for 2023 were reported at the twenty-fourth ACVVR meeting which took place in November 2022. In the USA, VARV is subject to the Select Agent Regulations (42 C.F.R. part 73).

Use of live variola virus to support less reactogenic vaccine development: continued evaluation of "third" generation vaccines

Investigators: Todd Smith, Audrey Matheny, Christine Hughes, Michael Townsend, Subbian S. Panayampalli, Whitni Davidson, and Christina Hutson
WHO Collaborating Center for Smallpox and other Poxvirus Infections Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Additional external collaborators: *Bavarian Nordic*
Yasuhiko Shinmura and Kengo Sonoda, KM Biologics Co., Ltd. (Successor of KAKETSU-KEN); and Masayuki Saijo from the National Institute of Infectious Diseases Japan

Variola virus (VARV) neutralization *in vitro* remains an informative surrogate measure of smallpox vaccine efficacy. The plaque reduction neutralization test (PRNT), which measures ability of sera to neutralize mature virus forms, has been used as a primary endpoint for the evaluation of vaccines. Our prior studies, using sera from *Vaccinia virus* (VACV)-vaccinees immunized using modified *Vaccinia virus* Ankara (MVA-BN recently licensed as JYNNEOS), ACAM2000, Dryvax or LC16m8 vaccines, have indicated neutralization end-point titers may differ when using different species of target viruses. Slight differences in orthopoxvirus antigens likely account for these differences.

Development of new vaccines has included significant focus on use of attenuated vaccine strains, such as MVA and LC16m8. These "third" generation vaccines, however, were never tested directly for efficacy against smallpox during the eradication campaign since most were developed towards the end or after smallpox eradication. We have found a statistically significant difference in neutralization titers of vaccinee serum when using different target viruses (VARV – heterologous target versus VACV – homologous target), which highlights the need for testing against VARV. Evaluation of the ability of sera to neutralize the mature virus form of VARV will provide a more informative surrogate measure of efficacy.

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 on results of an optimized VARV PRNT that showed no statistically significant difference between ACAM2000 and JYNNEOS, the US FDA approved JYNNEOS in 2019 for prevention of smallpox and monkeypox. All raw data of VARV PRNT assays has been archived for future availability and transparency. JYNNEOS has been the frontline vaccine administered in the USA during the 2022 global mpox outbreak.

For many years, CDC has been involved in a JYNNEOS vaccine study within the Democratic Republic of the the Congo, where Monkeypox virus (MPXV) Clade I is endemic. All serum from approximately 1000 participants vaccinated with JYNNEOS (liquid formulation, part 1 of the study which included blood collection time points of day 0, 14, 28, and 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 seroconversion in all assays when compared with prior data; responses in those with previous/ childhood vaccination 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 declines in responses.

Part 2 of the study using a lyophilized formulation of MVA-BN vaccine has concluded; the 12 month time-point was missed due to the COVID-19) pandemic. Part 3 in which some of the original participants will receive a boost to measure anamnestic response is underway.

As serum sample collection continues from the Democratic Republic of the Congo trial, neutralization against VARV *in vitro* can be assessed at additional time points beyond peak titre.

Understanding the longevity of humoral immunity will allow for efficient use of stockpiles, and development of effective utilization strategies, allotting more doses for protection of those most at risk. Valuable insights into the longevity of the immune response can be gained by screening samples for ability to neutralize VARV since differences in neutralization capacity have been documented according to the orthopoxvirus species used as the target within the PRNT assay.

In the USA, VARV is subject to the Select Agent Regulations (42 C.F.R part 73). Progress under this proposal and plans for 2023 were reported at the twenty-fourth ACVVR meeting which took place in November 2022.

Regeneration of noninfectious VARV materials for diagnostic development support

Christina Hutson, Kimberly Wilkins, Todd Smith, Chantal Kling, Audrey Matheny, Crystal Gigante, and Yu Li
WHO Collaborating Centre for Smallpox and other Poxvirus Infections, Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

As recently as 2021, new isolates of orthopoxvirus (OPXV) are being identified and can confound current diagnostic assays. The need to maintain variola virus (VARV) DNA and VARV antigen stocks at the WHO Collaborating Centre for Smallpox and other Poxvirus Infections remains important for future diagnostic development and validation. In the USA, 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). Greater understanding of the variability within VARV genomes will be instrumental to understand the sensitivity and specificity of nucleic-based diagnostic assays. 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 viruses occurred in 2020 (one), 2021 (two) and in 2022 (one). The compilation of VARV samples sequenced in previous years has been completed. Enhancing our database of VARV sequences and in-silico analysis capabilities will greatly improve understanding of limitations and ability to properly use medical countermeasures including diagnostics.

Diagnostic development has continued over the past year, focusing on validation of new reagents and/or equipment as technology advances to retain US FDA approval. The mpox outbreak has 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 US FDA approved OPXV assays. In 2022, we performed bridging studies to add an automated platform, an additional PCR platform and an extraction control to one of our FDA cleared PCR tests. Additionally, we performed safety testing of multiple lysis buffers being used in public health laboratories to ensure they inactivate OPXV. In 2023, we will work with our regulatory group at CDC to add additional platforms and reagents to our VARV specific PCR tests with regulatory approval.

During the past year we have worked with a private company to determine the performance of a commercially available OPXV test. Two Monkeypox virus (MPXV) strains, Clade II (West African) and Clade I (Congo Basin), were used to determine the limit of detection of an OPXV PCR kit. Two dilution series were prepared and tested in triplicates and octuplicates to determine the sensitivity range of both strains. The lowest concentration of each strain that could be detected with 95% confidence interval was considered as the LoD. Clade II MPXV was determined to have a LoD of 1.581 fg/ul and Clade I MPXV was determined to have a LoD of 5 fg/ul.

We have developed and improved wet lab and bioinformatic protocols for sequencing OPXV directly from clinical samples using the Oxford Nanopore MinION sequencer from 2019 to 2022. Using this approach, we have been able to determine OPXV species and MPXV clade within the first 40 minutes of sequencing. We have also made progress in scaling up our sequencing throughput and analysis in response to the 2022 mpox outbreak to hundreds of samples per week.

We have developed a direct metagenomics sequencing workflow from clinical samples that uses the Illumina NovaSeq platform and has been successful at producing whole genome sequences for mpox clinical samples with Ct <30. 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 have drafted a study plan with the company to test updated cartridges that will become commercially available. This testing will ensure both the OPXV generic test and MPXV specific test perform well after manufacture changes were made to the cartridges including ensuring that testing with VARV is still detected with the OPXV generic target. Our work with this instrument within 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 with independent validation being done in Kinshasa.

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. In pursuit of this, we analyzed the OPXV genome sequences and developed LAMP based OPXV generic assays, mpox generic assay and MPXV Clade II specific assays.

We are working on plans to validate these newly developed assays using the portable system developed at ASU. Initial validation with inactivated MPXV will be done at ASU. If this initial validation is successful, additional validation for sensitivity, specificity and with mock contrived specimens will occur at CDC. If successful, we would add a VARV specific target as part of this assay design.

Use of live variola virus to develop virus-specific protein-based diagnostic and detection assays

Michael Townsend, Todd Smith, Christina Hutson, and Subbian S. Panayampalli
WHO Collaborating Center for Smallpox and other Poxvirus Infections, Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

The ability to validate protein-based diagnostic capacity is critical for rapid detection and response to smallpox. During the 2014-2016 Ebola response in West Africa, the need for rapid and accurate diagnostic capacity in remote or central laboratories was critical for successful disease containment. Alternatively, point of care (POC) assays for antibody or antigen detection hastily adapted during epidemics have the potential to lead to public distrust and hamper global public health efforts if tests are inaccurate and insensitive. These events highlight the two sides of protein-based diagnostic assay development and use. This presentation provides results from utilization of variola virus (VARV) to validate several protein-based diagnostic assays for accuracy and sensitivity. In the USA, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73).

We have continued evaluating the use of a lateral flow assay (LFA) for field use in the Democratic Republic of the Congo, where Monkeypox virus (MPXV) infections are endemic. An IRB approved orthopoxvirus (OPXV)-generic POC test is being used in the DRC to detect MPXV in patient lesion samples. 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. The time from rash onset to test result was an average of 4.5 days compared to 30.3 days for processing in the national laboratory.

CDC has completed assessment of mAbs developed in prior years using a multiplexed Meso Scale Discovery (MSD) format assay with inactivated virus. Testing of antibody combinations as capture or detection mAbs in these assays showed that the use of a polyclonal antibody (pAb) inclusive test remains the most sensitive for detection of OPXVs and that incorporation of a mAb with pAb involves trade-offs between sensitivity and specificity. Highly specific VARV mAbs in combination with pAb have shown detection of VARV around 5 X 104 pfu/mL, but low-level cross-reactivity with other OPXVs was observed with these combinations at concentrations higher than 1 X 107 pfu/mL. Although high-specificity mAbs have been developed, final selection of a POC format and optimization of that assay is incomplete. In many assay formats, cross-reactivity has been observed so we are pursuing multiplexed detection to identify the highest sensitivity and specificity achievable.

Arizona State University (ASU) has developed a low-cost multiplex fluorescent LFA, which improved the sensitivity by 2 – 3 orders of magnitude compared to traditional LFA formats. We are evaluating fluorescent LFA using mAbs directed against *Vaccinia virus* or MPXV. Based on these results, we will later incorporate VARV detection mAbs in the multiplex assay. We also began exploring other platforms expected to improve antigen detection sensitivity.

In collaboration with Tetracore, we used a Luminex-based assay and observed a limit of detection as low as 1 X 103 pfu/mL for non-VARV OPXV. We are exploring this technology for multiplex-detection of OPXVs using species-specific mAbs. Both collaborations are on hold pending the resolution of the COVID-19 pandemic.

Abstracts from invited speakers

Update for brincidofovir oral tablets/oral suspension, smallpox (Vaccinia) vaccine, live, and vaccinia immune globulin intravenous (human)

Laura Cochrane
Emergent Biosolutions

On 26 September 2022, Emergent BioSolutions acquired brincidofovir oral tablets/oral suspension from Chimerix. Emergent is already the manufacturer of smallpox (vaccinia) vaccine (live) ACAM2000, and vaccinia immune globulin intravenous (human.)

Brincidofovir was approved by the US FDA on 4 June 2021, for the treatment of human smallpox disease in adult and paediatric patients, including neonates. Brincidofovir's mechanism of action and resistance profile differs from the approved treatment for smallpox (tecovirimat), allowing it to retain activity against strains resistant to the available approved therapy. In addition, brincidofovir adds another smallpox antiviral to the US Strategic National Stockpile, 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 US FDA post-marketing commitment which includes the evaluation of brincidofovir *in vitro*, against mutant strains of orthopoxviruses, and Emergent is also currently assessing a pharmacokinetic bioequivalence study.

The Public Health Agency of Canada has acquired brincidofovir under a special access program and Chimerix and Emergent are committed to the registration of brincidofovir in Canada.

As part of Emergent's broader smallpox portfolio, Vaccinia vaccine (ACAM2000) is a second-generation smallpox vaccine containing live *Vaccinia virus* and is indicated for the active immunization against smallpox disease for persons determined to be at high risk for smallpox infection. It is currently licensed by the US FDA, Australia Therapeutics Goods Administration (TGA) and Singapore Health Sciences Authority (HSA).

As part of the US FDA post-licensure marketing commitments for vaccinia vaccine, several studies have already been completed for smallpox (vaccinia) vaccine, live and a prospective safety surveillance[1] and retrospective surveillance[2] study in US military personnel who were administered vaccinia vaccine was conducted. Also included in the post-licensure marketing commitments was an investigational new drug (IND) study of vaccination with vaccinia vaccine in previously vaccinated plasma donors. An EAP in the USA for vaccinia vaccine is currently available for vaccination of individuals at risk for non-variola orthopoxvirus infections during an outbreak.

1. Faix DJ, Gordon DM, Perry LN et al. Prospective safety surveillance study of ACAM2000 smallpox vaccine in deploying military personnel. Vaccine. 2020; 38(46):7323-30, ISSN 0264-410X. Available at: https://doi.org/10.1016/j.vaccine.2020.09.037
2. Decker MD, Garman PM, Hughes H et al. Enhanced safety surveillance study of ACAM2000 smallpox vaccine among US military service members. Vaccine. 2021; 39(39):5541. Available at: https://doi.org/10.1016/j.vaccine.2021.08.041. Epub 2021 Aug 26. PMID: 34454787.Licensing and marketing update for brincidofovir.

Vaccinia immune globulin intravenous (human), contains antibodies to *Vaccinia virus* from healthy, screened donors immunized with vaccinia vaccine for the treatment of complications due to vaccinia vaccination, including eczema vaccinatum, progressive vaccinia, severe generalized vaccinia, vaccinia infections in individuals who have skin conditions, and aberrant infections induced by *Vaccinia virus* (except in cases of isolated keratitis). This product is currently licensed by the US FDA and Health Canada. An ongoing US Centers for Disease Control and Prevention (CDC) expanded access IND protocol for vaccinia immune globulin intravenous (human) in the treatment of human orthopoxvirus infections during an outbreak is currently in place, and US FDA post-marketing commitments to confirm clinical outcomes of this treatment of smallpox vaccination complications or vaccinia infections.

Continued development of tpoxx (tecovirimat) and its deployment and use during the ongoing monkeypox epidemic

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

Updates were provided on new regulatory approvals of oral tecovirimat and IV tecovirimat, as well as development updates on a pediatric formulation and on efforts to obtain regulatory approval for a post-exposure prophylaxis indication for oral tecovirimat. The year 2022 saw an unexpected global outbreak of mpox. Fortunately, SIGA had a stockpile of tecovirimat available and animal data to support its efficacy against mpox. The drug has been widely and successfully used around the world to treat mpox-infected patients. Even as this situation continues, SIGA is supporting several observational and placebo-controlled clinical trials to substantiate the safety and efficacy of the drug to hopefully allow additional regulatory approvals and improve patient access

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 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. Currently, LC16 vaccine is intended for emergency use and has been stockpiled in Japan as a safeguard against potential bioterrorism with smallpox virus.

In August 2022, the indication of LC16 vaccine was updated to "Prevention of smallpox and mpox" (underline denotes additions)" [1] and the approved shelf-life at -20° C storage was updated from 4 to 10 years based on each evidence data.

The "Vaccines and immunization for monkeypox: Interim guidance" developed by the WHO supports the clinical usefulness of smallpox vaccines in prevention of mpox. In the guidance, smallpox vaccine LC16 is listed as one of the smallpox vaccines to be used for prevention of mpox. Globally, the LC16 vaccine is the sole smallpox and mpox vaccine that can be administered to children and adults.

1 Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 "KMB" KM Biologics Co., Ltd. Review Report. Review Report. 21 July 2022. Available at: https://www.pmda.go.jp/files/000247943.pdf

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

Florian Lienert
Bavarian Nordic

In 2022, the indication of MVA-BN in the EU and United Kingdom has been expanded to include active immunization against smallpox, mpox and disease caused by *Vaccinia virus* in adults. An expanded shelf-life of the vaccine has been approved in the EU and USA.

MVA-BN is being used by many countries responding to the ongoing mpox outbreak in non-endemic countries. More than 70 countries have access to MVA-BN, including countries in Latin America and the Caribbean through an agreement with the PAHO. Based on publicly available information up to 28 November, more than 1.3 million doses of MVA-BN have been administered since the May 2022.

Initial reports on the effectiveness of MVA-BN in preventing mpox have been published.

The US CDC reported that the mpox incidence in unvaccinated persons was 14 times higher than in persons vaccinated with 1 dose of MVA-BN. A not yet peer-reviewed preprint article describes a cohort study that included all Israel Clalit Health Services members eligible for MVA-BN as per country guidelines (n= 2,092). In total 1 068 (51%) subjects were vaccinated subcutaneously with MVA-BN and completed at least 25 days of follow-up. Mpox infections occurred in 16 unvaccinated persons and 5 vaccinated persons. Vaccine effectiveness was estimated at 87% (95% CI: 60%-95%). Another not yet peer-reviewed preprint article reports 78% (95% CI: 54%-89%) effectiveness ≥14 days after a single dose of MVA-BN in England using the case-coverage method.

Several additional studies on the effectiveness, immunogenicity and safety profile of MVA-BN in real-world use are ongoing.

Potency testing of first generation smallpox vaccines from the WHO stockpiles

Harry Venema, Gabriel Goderski, Carla Nijhuis, Harry van Dijken, Leon Schutte and Jørgen de Jonge
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (Kingdom of the).

As the WHO Collaborating Centre for Smallpox Vaccines, we are responsible for the potency testing of smallpox vaccines from the WHO emergency reserves, as described in the terms of reference. In 2022, we vaccinated and trained new personnel and set up a new laboratory to secure future potency testing. We performed the test on vaccine samples received from the WHO in September 2022.

The potency of first generation smallpox vaccines is determined by titration on the chorioallantoic membrane (CAM) of embryonated chicken eggs. Briefly, a small window is cut out of the egg to expose the CAM. Three virus dilutions (5 logs) are administered in 9 replicates with the middle dilution targeting 8log pox forming units (pfu) per mL. After

a 2 day incubation at 37 °C, the CAM was recovered and fixed in formalin, after which the pox were counted. Seven out of the nine batches had a titer of >8log pfu/mL and two batches were slightly below 8log pfu/mL (7.88 pfu/mL).

The potency of four out of the five batches tested since 2003 remained stable and in one batch the titer declined slightly. Compared to vaccines held in the Netherlands (Kingdom of the), the titers are similar (above 8log pfu/mL). Thus, the vaccines remain stable and comply to the minimal titer required for vaccination with minor exceptions.

Antivirals and vaccine regulatory status

Lorna Leal Alexander, **European Medicines Agency (EMA)**

Tecovirimat

Tecovirimat (SIGA) is the only antiviral approved by the European Medicines Agency (EMA) for the treatment of smallpox, monkeypox and *Vaccinia virus* infection. This approval was granted on 6 January 2022, under exceptional circumstances as the applicant was unable to provide comprehensive data on the efficacy and safety under normal conditions of use due to the epidemiological situation.

Other antivirals used for poxvirus treatment

Cidofovir (Gilead) – Approved in 1997 for cytomegalovirus-caused retinitis treatment. The marketing authorization was withdrawn from use in EU at the request of the marketing authorization holder; however the product is still available in several EU Member States.

Brincidofovir (Chimerix – Emergent Biosolutions) – Orphan designation was granted by the European Commission in 2016, for the prevention of cytomegalovirus disease, the treatment of adenovirus infection in immunocompromised patients, and the treatment of smallpox.

EMA efforts in data collection for mpox treatment

Several initiatives in different EU Member states have been presented and discussed. For example, the MOSAIC study: A cohort study collecting harmonized data in patients treated or not treated with tecovirimat for mpox, conducted by the Oxford University, ANRS and HUG; and the EPOXI trial: a European randomized clinical trial that aims to find the best treatment for mpox, conducted by Ecraid. The EMA has also contributed to the RCT study design developed by WHO (CORE protocol) and chairs the WHO Target Product Profiles (TPP) Working Group for mpox therapeutics.

Modified Vaccinia Ankara vaccine by Bavarian Nordic (MVA-BN)

MVA-BN (IMVANEX) is the only vaccine approved by EMA for active immunization against smallpox disease, under exceptional circumstances. On 22 July 2022, there was extension of indication to active immunization against smallpox, mpox and disease caused by *Vaccinia virus* in adults. Following the Public Health Emergency of International Concern (PHEIC) declaration, with the aim to minimize shortages and in view of the increasing number of cases, the EU Emergency Task Force (ETF) issued a recommendation to support

vaccination strategies for antigen sparing (intradermal (ID) delivery of a fractional dose). This opinion does not reflect the assessment of quality, safety and efficacy necessary to achieve a positive benefit-risk balance needed for an approval.

Other vaccines used for poxviruses immunization

LC16m8 (KM Biologics Co., Ltd) – The ETF has performed a preliminary assessment of the available non-clinical and clinical data. EMA is monitoring and discussing with stakeholders the vaccines under development.

EMA efforts in data collection for mpox vaccines

The SEMVAc study, that evaluates the effectiveness of MVA-BN vaccination against mpox infection in at-risk individuals in Germany has been considered and funded by the recently established Vaccine Monitoring Platform (VMP), a joint collaboration between EMA and the European Center for Disease Control (ECDC), for the timely generation of independent, real-world evidence of vaccines in use in EU/EEA immunisation programmes. EMA has a strong collaboration with VACCELERATE, an EU network for vaccine clinical trials, and serves as a facilitator in the EU network for paediatric data collection.

Regulatory status of antivirals for smallpox and mpox: US FDA perspective

Patrick Harrington
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 US FDA for the treatment of smallpox. These drugs were approved based on the US FDA "Animal Rule", which provides a pathway for approval of drugs and biological products when it is not ethical or feasible to conduct efficacy studies in humans. Tecovirimat and brincidofovir are not US FDA approved for the treatment of mpox as US FDA has determined that the Animal Rule is not a viable regulatory pathway to approve drugs for the treatment of monkeypox, as it is both feasible and ethical to conduct clinical trials in humans. In the USA, tecovirimat is available for 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 EAP sponsored by the US CDC. Brincidofovir is available via single-patient emergency use Investigational New Drug Applications for patients who meet specific eligibility criteria.

Cidofovir also is not US FDA approved for treatment of mpox; it is sometimes used off-label for monkeypox or other orthopoxvirus infections. It is the US FDA position that randomized, controlled clinical trials are the best approach to establish efficacy and safety for the treatment of mpox in humans, to understand how best to use these drugs, and to identify limitations of treatments that could guide further drug development. US FDA will continue to work with sponsors, government agencies, and the broader public health community to facilitate the clinical evaluation of tecovirimat and brincidofovir for treatment of mpox, and to support further drug development for mpox and smallpox. Information on tecovirimat and brincidofovir and how to access these drugs in the USA. for the treatment of mpox is provided on the US FDA Monkeypox Response webpage: https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/fda-monkeypox-response.

Monkeypox Diagnostics Target Product Profile (TPP) Development

Jilian A. Sacks, **Lab Pillar of the WHO Global mpox response Incident Management Team**

Since early May 2022, cases of mpox – caused by infection with Monkeypox virus (MPXV) – have been reported from countries that have not previously reported cases and continue to be reported in West and Central African countries. Increased circulation of MPXV globally has increased demand for diagnostics, prompted rapid development of commercial kits, and driven expansion of networks of laboratories and health facilities offering diagnosis. However, a clear need for more simplified, automated and/or accessible assays has been identified, including those that can enable testing at decentralized sites outside the laboratory. In response, to increase access to quality-assured, accurate and affordable mpox diagnosis, WHO has initiated the process of drafting target product profiles (TPP) for *in vitro* diagnostics for mpox. TPPs are planning tools for the development of health products, which specify the intended use, target populations and desired attributes of products. The primary target audience for the TPPs are manufacturers, suppliers, and researchers developing new assays; as well as countries and agencies evaluating and/or selecting assays for procurement and use for mpox testing. An expert consultation process was initiated in 2022 and two TPPs are being drafted. The draft of the two TPPs will be posted for public consultation before finalization and release.

Evaluation of eleven commercially available PCR kits for detection of Monkeypox virus DNA

Andreas Nitsche, **Robert Koch Institute**

Before the international spread of monkeypox in May 2022, PCR kits for the detection of orthopoxviruses, and specifically Monkeypox virus, were rarely available. Here we describe the evaluation of 11 recently developed commercially available PCR kits for the detection of Monkeypox virus DNA. All tested kits are currently intended for research use only and clinical performance still needs to be assessed in more detail. However, all were found to be suitable for detection of Monkeypox virus, with variations in specificity rather than sensitivity.

EPIDEMIC
AND PANDEMIC
PREVENTION AND
PREPAREDNESS

9789240076310