WHO ADVISORY COMMITTEE ON VARIOLA VIRUS RESEARCH

REPORT OF THE TWENTY-THIRD MEETING

VIRTUAL MEETING, 3–4 NOVEMBER 2021

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WHO Advisory Committee on Variola Virus Research: report of the twenty-third meeting, virtual meeting, 3–4 November 2021

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

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ABBREVIATIONS

ACVVR	Advisory Committee on Variola Virus Research
CDC	United States Centers for Disease Control and Prevention
COVID-19	coronavirus disease 2019
ELISA	enzyme-linked immunosorbent assay
EV	enveloped virion
FBRI SRC VB VECTOR	Federal Service for Surveillance on Consumer Rights Protection and Human Well-being State Research Center of Virology and Biotechnology
IMV	intracellular mature virion
IND	investigational new drug
mAb	monoclonal antibody
MVA	modified vaccinia Ankara vaccine
NIOCH	Novosibirsk Institute of Organic Chemistry
PCR	polymerase chain reaction
PFU	plaque-forming unit
PRNT	plaque reduction neutralization test
SAGE	WHO Strategic Advisory Group of Experts on Immunization
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
VARV	variola virus
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-third meeting on 3–4 November 2021 by video conference. The recommendations of the Committee are summarized in the report.

Variola virus repositories

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

Research update

The Committee received reports on progress of approved research using variola virus. Eighty-eight isolates remain to be sequenced at VECTOR. While sequencing of isolates for which epidemiological information was available had been completed at CDC, there remained 15 specimens without these data to be sequenced. Both centres indicated the remaining strains could be sequenced within the next 12 to 24 months. The Committee recommended continuation of previously approved projects and approval of one project amendment.

Antiviral agents

Smallpox antiviral studies had continued and two compounds were now approved for the treatment of smallpox. As anticipated at the meeting, oral tecovirimat was also approved in the European Union (EU) for treatment of smallpox, monkeypox, cowpox and vaccinia complications. Development of paediatric and intravenous formulations of tecovirimat continued. A clinical study of oral tecovirimat to treat monkeypox in field settings had begun in the Central African Republic. The oral antiviral therapeutic 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 in the Russian Federation showed that assessed oral regimens were safe; licensure was expected shortly. Testing new chemical compounds with high selectivity against variola virus in vitro would continue. The Committee welcomed a report on potential new molecular targets for orthopoxvirus antiviral treatment. CDC proposed further study of a humanized mouse model for assessing antiviral treatments. Both WHO collaborating centres continued to explore monoclonal antibodies and a Russian Federation patent for a chimeric antibody was expected in 2022. At CDC, in collaboration with partners, four new monoclonal antibody mixes appeared to be effective against intracellular and enveloped virion forms of variola and monkeypox viruses.

Vaccines

Studies with modified vaccinia Ankara vaccine developed by Bavarian Nordic (MVA-BN) continued in different contexts, including for the prevention of human monkeypox 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

indications for the vaccine to include prevention of monkeypox and other orthopoxviruses for persons at risk. VECTOR continued development of VACΔ6, a fourth-generation attenuated vaccinia vaccine, and Phase II and III clinical trials were conducted; licensure in the Russian Federation was anticipated for 2022.

Diagnostics

VECTOR continued to assess an immunochemical test kit for rapid, point-of-care detection of orthopoxviruses; the assay was specific and easy to use and would, with further development, be suitable for field settings. CDC continued to improve both nucleic acid-based and protein-based rapid diagnostic tests; detection of variola in a lateral flow protein-based assay had shown promising results. The Committee recommended continuing work towards developing test technology that would not require the use of live virus to validate; advances observed during the coronavirus disease 2019 (COVID-19) pandemic could serve as a basis from which to further develop diagnostic techniques for orthopoxviruses. The Committee also recommended a roadmap be established to leverage advances in smallpox diagnostics for the development of point-of-care diagnostics for monkeypox in low-resource settings and their deployment in a reliable and equitable manner.

Lessons learned from the COVID-19 pandemic

The Committee discussed the implications for preparedness for smallpox-like events reflected by the ongoing COVID-19 pandemic. The Committee noted how quickly diagnostics and vaccines could be developed and deployed when resources and political will were abundant. This rapidity was also due to the fact that the genetic sequence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had been shared worldwide. It was noted that in one country SARS-CoV-2 had been reconstructed in a laboratory from the viral genome sequence before the first case of COVID-19 had been reported, highlighting the benefits of synthetic biology technologies for accelerated development of diagnostics as well as the oft-described potential risks. Lessons learned about clinical care during the COVID-19 pandemic were also discussed.

Conclusion

The Committee noted that with a second antiviral therapeutic approved, the objectives of the research programme endorsed by the World Health Assembly were being met. Conditions under which the various treatments would be deployed would require consideration. It was further noted that resources developed for smallpox preparedness may be key to controlling the emergence of monkeypox, illustrating the potential near-term public health benefit arising from the variola virus research programme. The Committee suggested that the lessons learned from the ongoing COVID-19 pandemic continue to inspire development efforts for rapid, point-of-care diagnostics. Other recommendations offered by the Committee are summarized in the report.

MEETING PROCEEDINGS

The twenty-third 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, 3–4 November 2021. The meeting was co-chaired by Dr Andreas Nitsche and 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. The meeting took place via videoconference.

The objectives of the meeting were to:

- review progress of approved research with live variola virus (VARV); and
- update the research programme and recommendations for 2020–2022.

Dr Sylvie Briand, Director, WHO Global Infectious Hazards Preparedness Department, welcomed all participants and spoke of the ongoing coronavirus disease 2019 (COVID-19) pandemic, which had caused over 5 million deaths worldwide at the time of the meeting. The COVID-19 pandemic had compelled the global community to innovate and build new partnerships, engage in collective efforts to licence numerous vaccines for COVID-19, and establish an epidemic and pandemic intelligence hub in Berlin among other initiatives. Dr Briand noted that ecosystems worldwide were under threat due to climate change, leading to rising risks from vector-borne diseases and zoonoses. While smallpox had been eradicated, re-emergence was still a risk, and the global community needed to face that risk together. Therefore the work of the Committee remained critical. The issue of the destruction of live variola virus stocks would be discussed again soon by the World Health Assembly. The discussion would be informed by the COVID-19 pandemic and the global response to it.

The WHO transformation, begun in 2019, had led to efforts to foster greater transparency at WHO and new ways of working, including how expert committees were convened. Dr Briand thanked Committee members for their work and chairpersons for facilitating the discussions.

In her remarks, **Dr Maria Van Kerkhove**, Head, Emerging Diseases and Zoonoses Unit, began by thanking Committee members for their research on variola virus and other orthopoxviruses. During the COVID-19 pandemic there had been strong collaboration among scientists, and innovation in online communication had allowed more stakeholders to join research and policy discussions. The scientific community had learned the importance of preparedness for known pathogens and new ones. Dr Van Kerkhove commented that the pandemic would continue to disrupt lives in myriad ways. The extensive political attention and media coverage on the pandemic could be seen as a catalytic moment for public health, including funding research and development, expanding surveillance and bolstering preparedness for emerging pathogens.

Dr Andreas Nitsche, chairperson, introduced the agenda for day 1: updates from the two WHO collaborating centres on their variola virus and DNA collections and on the progress of research on antivirals and monoclonal antibodies (mAbs). He reminded Committee

members that the original focus of the Committee had been the creation of vaccines, antivirals and diagnostics as well as advising on the timing of destruction of variola virus stocks. In light of the COVID-19 pandemic and response, 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.

SECRETARIAT REPORT

Dr Rosamund Lewis, Head of the Smallpox Secretariat, WHO Health Emergencies Programme, welcomed all participants, took roll call and thanked members who had left in 2021: Professor Oyewale Tomori and Dr Aissatou Toure. Dr Lewis reminded members that their terms of reference had been updated[1] in line with new WHO requirements on convening of expert advisory groups, and that members served in their personal capacity as experts. The mandate of the Committee continued unchanged, namely to advise on variola virus research and biosafety inspections and to make recommendations in line with World Health Assembly resolutions WHA52.10, WHA55.16 and WHA60.1 as well as decision WHA64(11), which authorized temporary retention of variola virus stocks, subject to annual review. Committee members from the collaborating centres would become permanent representatives to the Committee. Interests declared by other Committee members concerned working for their respective government agencies.[2]

Dr Lewis introduced the report from the WHO Smallpox Secretariat. The term of the research programme developed following the Seventy-second World Health Assembly and published in previous reports[3] was for 2020–2022. As the COVID-19 pandemic had affected completion of approved projects, ongoing research would be presented as progress reports for discussion.

An update was presented on orthopoxvirus events over the past year. Two cases of Alaskapox had been confirmed in the United States of America bringing the number of human infections with this newly described orthopoxvirus to four. In June 2021, monkeypox had been diagnosed in the United Kingdom of Great Britain and Northern Ireland in a traveller from Nigeria, leading to a secondary and tertiary case in the same family. During this event, health workers had been offered the modified vaccinia Ankara vaccine by Bavarian Nordic (MVA-BN) monkeypox vaccine. In July and November 2021, two further unrelated cases of monkeypox presented to health services in the USA upon return from travel to Nigeria. Details of each event had been published.[4,5,6] Outbreaks continued to be reported in the Democratic Republic of the Congo.

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1 Which can be found at: tors-acvvr_august2021.pdf (who.int).

2 This interest concerned the following: Supamit Chunsuttiwatt, George Korch, Andreas Nitsche, Wenjie Tan and David Ulaeto, as well as Inger Damon and Rinat Maksyutov as permanent representatives.

3 Advisory Committee for Variola virus Research [website]. Geneva: World Health Organization, 2022 (https://www.who.int/groups/who-advisory-committee-on-variola-virus-research/meeting-documents, accessed 23 February 2022); summaries published separately.

4 Disease Outbreak News. Monkeypox – United Kingdom of Great Britain and Northern Ireland [website]. Geneva: World Health Organization; 2021 (https://www.who.int/emergencies/disease-outbreak-news/item/monkeypox---united-kingdom-of-great-britain-and-northern-ireland, accessed 23 February 2022).

5 Disease Outbreak News. Monkeypox – United States of America [website]. Geneva: World Health Organization, 27 July 2021 (https://www.who.int/emergencies/disease-outbreak-news/item/monkeypox---the-united-states-of-america, accessed 23 February 2022).

6 Disease Outbreak News. Monkeypox – United States of America [website]. Geneva: World Health Organization; 25 November 2021 (https://www.who.int/emergencies/disease-outbreak-news/item/2021-DON344, accessed 23 February 2022).

Dr Lewis provided an update on the WHO orthopoxvirus laboratory survey undertaken in 2021 which would help to map global diagnostic capacity for orthopoxviruses and assess whether laboratory staff had access to vaccine or treatment options for orthopoxvirus infections. WHO thanked Committee members who had reviewed, piloted, responded and helped to disseminate the survey. Monkeypox outbreak response training for health professionals developed by WHO would soon be available in English and French on the online training platform OpenWHO.[7] The Secretariat extended thanks to all who had assisted in developing these courses.

To ensure that public health benefit continue to accrue from the work of the Committee, it was reported that updated guidance on the use of smallpox vaccines would be sought from the WHO Strategic Advisory Group of Experts on Immunization (SAGE) in 2022. Topics would include the use of vaccines for smallpox preparedness and response; occupational health and safety for personnel working with or potentially exposed to orthopoxviruses; and monkeypox prevention and control. In discussion, the Committee endorsed the reconvening of the Ad Hoc Committee on Orthopoxviruses, and/or the creation of an informal working group, to prepare for the consultation with SAGE.

Continuing the Secretariat report, **Dr Kazunobu Kojima**, Technical Officer, Health Emergencies Programme, shared that as reported to the World Health Assembly in May 2021, biosafety and biosecurity inspections of the two WHO variola virus repositories and research centres had been rescheduled due to the COVID-19 pandemic. They were planned for December 2021 at VECTOR[8] and May 2022 at CDC.

Ms Alexandra Hill, Technical Officer, Health Emergencies Programme, also shared an update on preparedness planning for a smallpox event. WHO had taken part in exercises related to orthopoxviruses, one in collaboration with external partners[9] in March 2021, and an internal exercise with two WHO regions in October 2021. WHO continues to hold 2.8 million doses of mainly first-generation smallpox vaccine. Stock inventory and potency testing were planned for January 2022. The pledged stock consisted of 27 million doses, and was under review with participating countries. A project conducted with Johns Hopkins University over 2020–2021 had included a literature review of past outbreaks; a survey of smallpox vaccine and antivirals production capacity; and development of possible vaccine deployment scenarios. While the findings were under review, it was noted that one key finding was that overall smallpox vaccine production capacity was declining worldwide.

[7] Monkeypox epidemiology, preparedness and response. OpenWHO [website]. Geneva: World Health Organization; 2022 (https://openwho.org/courses/monkeypox-intermediate, accessed 23 February 2022).

[8] The planned December 2021 mission was since rescheduled to 2022 to ensure avaibility of inspectors.

[9] The Munich Security Conference and the Nuclear Threat Initiative. Yassif JM, O'Prey KP, Isaac CR. Strengthening global systems to prevent and respond to high-consequence biological threats. Washington (DC): Nuclear Threat Initiative; 2021 (https://www.nti.org/analysis/articles/strengthening-global-systems-to-prevent-and-respond-to-high-consequence-biological-threats/, accessed 23 February 2022).

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 2020. Eighty-eight isolates remain to be sequenced.

The projects requiring use of variola virus approved by WHO to be conducted in 2021 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. use of live variola virus to evaluate 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. With respect to use of variola virus, Dr Maksyutov reported that the study supporting development of a fourth-generation vaccine was conducted in 2021, and other projects listed above were scheduled to be resumed in 2022, following WHO approval of continuation of the projects proposed by VECTOR.

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 Victoria Olson 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. The variola virus collection comprised 360 non-identical isolates stored in two long-term repositories.[10] CDC had completed initial assembly and phylogenetic analysis of all isolates with extracted DNA to date. Some additional sequencing may be required if there is insufficient coverage for any of those isolates. Fifteen isolates without good epidemiological information remain to be sequenced. In April 2021, a failure of one of the long-term repositories had necessitated

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

movement of the samples to another freezer. The repository freezer had been repaired and samples had been returned to long-term storage on 2 August 2021. WHO had been informed in writing to document this movement, which followed established protocols.

The projects requiring use of live variola virus approved by WHO for conduct in 2021 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. determine whether mice are a suitable animal model for human smallpox (Partially approved 2019; Approved 2020);
5. evaluate antivirals (monoclonal biologics) against variola virus (Approved 2019);
6. support less reactogenic vaccine development: continued evaluation of "third" generation vaccines (Approved 2019); and
7. develop protein based (and DNA-based) diagnostic and detection assays specific for variola virus (Approved 2019).

In total, variola virus had been used 28 times: two uses for nucleic acid-based diagnostic assays; one for protein-based diagnostic assays; and 25 for antiviral therapeutic development with mAbs.

Discussion: reports from the WHO collaborating centres

Over recent years full genome sequencing of isolates of variola virus held in the two WHO collaborating centre repositories had progressed. The Committee agreed that knowledge of the full genome sequences of all variola virus isolates held in the two collaborating centre repositories was important not only to improve epidemiological understanding of smallpox infections, but also for in silico assessment (i.e. performed on a computer or by computer simulation) of diagnostic polymerase chain reaction (PCR) and other assays in terms of sensitivity and specificity, as well as the identification of variola virus variants that may be resistant to antiviral drugs. If new antiviral drugs against variola virus were to be evaluated, the knowledge of the full genome sequences would be helpful to pre-select candidate drugs. The same would hold true for new modern molecular techniques to be evaluated for variola virus diagnostics.

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 had been performed on specimens with sufficient metadata for epidemiological analysis and was to be completed by 2022. The collaborating centres estimated that sequencing of all remaining isolates, including those without epidemiological metadata, could be accomplished by the end of 2022 (at CDC) or 2023 (at VECTOR). If sequencing of all remaining specimens were not possible under an agreed time frame, the WHO collaborating centres were encouraged to define criteria to allow prioritization of the sequencing order, 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. A clinical trial had been conducted to determine safety, tolerability and pharmokinetics of the drug. In July 2020, the ministry of health of the Russian Federation had approved Phase I trials to begin in September 2020 and 90 persons (aged 18–50 years) had been recruited. Six groups of 15 participants each received various dose regimens as single doses for one day (200 mg, 600 mg and 1200 mg) or daily doses for a period of six days each of 200 mg, 600 mg and 1200 mg (split into two doses).

Safety studies included assessment of side effects and standard clinical assays 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 if 200 or 600 mg daily, no adverse events were reported. One volunteer reported chest and abdominal pain on days 2 and 5 of the 1200 mg daily regimen, which however was not linked to any specific diagnosis or change in clinical parameters. The researchers concluded that the Phase I clinical trial as previously and presently reported had demonstrated that the smallpox 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.

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 and prevention in a project approved in 2019. The goal of this study had been to test the antiviral activity of novel chemical compounds using surrogate orthopoxviruses, in order to identify the most effective drugs for further efficacy assessment against live variola virus in vitro. These compounds belonged to classes of chemical compounds that had not yet been tested for activity against orthopoxviruses; between 2019 and 2021, 400 compounds 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[11] were used as reference compounds.

Preliminary studies conducted had led to identification of 15 compounds with high selectivity indices. Selectivity index scores (i.e. TC50/IC50[12]) ranged between 792 (for the compound PANI-62 with derivatives containing ester or amide fragments) and 18 166 (for compound MS-252 containing fragments of camphor, borneol or adamantine). These 15 compounds would be tested further using live variola virus. It is proposed to conduct this research in 2022.

[11] 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.

[12] TC50 refers to the concentration at which 50% of cells in an uninfected monolayer are destroyed; IC50 refers to an inhibitory concentration at which 50% of cells are retained in the infected monolayer.

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 United States Food and Drug Administration (FDA) to further assess sensitivity of variola virus strains to tecovirimat, and approved in 2019. Seven of the ten identified F13L amino acid variants were available and tested sensitive to tecovirimat (as reported in previous meetings). The remaining three F13L amino acid variants will be expressed in stable cell lines to evaluate sensitivity to tecovirimat during infection with vaccinia virus not expressing the F13L protein (VACVΔF13L). If additional sequences become available in 2022, newly sequenced isolates would be analysed in order to determine if additional F13L variants exist. For newly sequenced variola virus isolates with F13 amino acid variants which are unique and not previously tested, the isolate would be propagated to test for tecovirimat sensitivity.

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

Dr Todd Smith presented an update to the research project approved in 2019 to assess the effectiveness of antiviral therapeutic ST-357, a promising antiviral discussed in previous meetings. Due to challenges encountered with solubility characteristics of ST-357, this project had been delayed until additional analogues become available for assessment. Further research would screen ST-357 derivatives against orthopoxviruses. This in vitro research planned for 2022 would aim to determine the half maximal effective concentration (EC_{50})[13] of ST-357 analogues to identify pre-clinical candidates and may involve variola virus propagation (if needed) to attain material for antiviral screening.

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

Dr Christina Hutson shared an update on this project.[14] 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 mice were infected with variola virus and treated with tecovirimat, an established smallpox antiviral drug. Hu-CD34 mice had been tested first due to limited availability of hu-BLT mice.[15] All mice in two groups were infected with variola virus and followed for 42 days, with one group receiving 14 days of tecovirimat starting on days 0, 1 or 2, and the other group receiving no treatment. Initial results had shown tecovirimat treatment to be fully effective only if begun on Day 0 of virus challenge, with 50% mortality appearing with later treatment. All mice in the group without tecovirimat treatment succumbed to infection.

Continuation of the project was proposed to complete biomarker analysis of specimens from prior phases of this study and complete testing and analysis for the CD34 tecovirimat study. The Collaborating Centre now invited the Committee to consider a proposal to examine the more immunocompetent hu-BLT mice (if they could be made available) with

[13] EC_{50} refers to the effective concentration required to obtain 50% of the maximum possible effect.
[14] This project had been partially approved in 2019 and further approved in 2020.
[15] BLT humanized mice have received transplants of human haematopoietic stem cells, liver and thymus; BLT refers to the tissues grafted, namely bone marrow, liver and thymus.

the same 14-day treatment protocol or to repeat the CD34 mouse study with a longer (21-day) treatment period. Committee members were generally in favour of either option subject to WHO approval.[16] A point was made to consider studying which human cells were needed to allow virus to spread and cause morbidity and mortality within the model to more closely resemble human infection.

New perspectives: restriction of orthopoxviruses by TRIM5α

Professor Geoffrey Smith presented research on a potential new target for orthopoxvirus antiviral treatment. It was found that production of a protein known as TRIM5α[17] by human cells in vitro was downregulated after infection of the cells by vaccinia virus. In cells in which the TRIM5α gene was knocked out, vaccinia virus plaque size and virus titre increased, confirming a role for TRIM5α in restricting vaccinia virus infection. Prior reports had shown that vaccinia virus incorporated the protein cyclophilin A into the virion core, and recent studies had shown that when the Cyclophilin A gene was knocked out, vaccinia virus plaque size and virus titre were reduced. It was also known that the immunosuppressive drug cyclosporin A, an antagonist of cyclophilin A, inhibited vaccinia virus replication and reduced vaccinia virus plaque size.

The research team had confirmed these observations and shown that the inhibition of vaccinia virus replication by cyclosporin A was TRIM5-dependent. Collectively, these results had shown that: i) human TRIM5 restricts vaccinia virus replication; and ii) vaccinia virus counters this restriction by incorporation of Cyclophilin A into virions. Cyclosporin A could counter these viral defenses. Should research show similar results for monkeypox or other orthopoxviruses, it could lead to potential targets for variola virus antivirals which would differ from those of currently licenced smallpox antiviral agents. It is therefore planned to assess whether the same mechanisms pertain for monkeypox virus in vitro; plaque size and viral titres would be analysed.

Tecovirimat and ST-357: licensing and production update

Dr Dennis Hruby provided further updates on tecovirimat and ST-357. An application to Health Canada for oral tecovirimat was under review.[18] The indication would be for treatment of smallpox only, as in the USA. Pending approval of oral tecovirimat by the European Medicines Agency was expected to include marketing authorization for treatment of smallpox, monkeypox, cowpox and vaccinia complications.

Ongoing development was reported for oral tecovirimat: i) a post-marketing pharmacokinetic clinical study in volunteers weighing more than 120 kg had been completed – this was expected to lead to a label change on dose recommendations for this adult group;[19] ii) two powder formulations for children would undergo pharmacokinetic clinical studies; and iii) the United States Department of Defense would be engaging in studies of tecovirimat

[16] Following this meeting, the collaborating centre submitted a request for project amendment to WHO. The recommendations of the Committee for this amended proposal submitted are recorded in Annex 3.

[17] Tripartite motif protein 5 (TRIM5) – TRIM5α is a host restriction factor, known for its role in restricting replication of retroviruses, such as HIV-1, but not known to have antiviral activity against DNA viruses.

[18] Tecovirimat was approved on 1 December 2021 by Health Canada and on 10 January 2022 by the European Medicines Agency. Marketing authorizations were approved as requested and outlined in the text.

[19] The recommended dose of tecovirimat for adults 40 kg and above is 600 mg twice a day for 14 days. For adults of 120 kg and above, the dose would be 600 mg three times a day for 15 days.

for post-exposure prophylaxis, to commence in late 2021. Regulatory filing with the FDA for an intravenous formulation was under review. In 2021, intravenous tecovirimat had been used to treat a poxvirus-infected patient, with no complications. Guidelines for switching from intravenous to oral tecovirimat were being developed.

To bolster information on the use of oral tecovirimat to treat monkeypox in field situations, a clinical study had begun in the Central African Republic, to be managed by Oxford University in collaboration with the Institut Pasteur de Bangui.

Efforts continue to advance the proposed antiviral drug, ST-357. These efforts include computer modelling to predict analogues with improved drug characteristics and developing analogues of ST-357 with better solubility profiles.

Brincidofovir: licensing and production update

Dr Odin Naderer provided an update on the licensing and production of brincidofovir. In June 2021, the FDA had approved the short-course therapeutic in tablet and oral suspension formulations for the treatment of smallpox disease in adults and children, including neonates and infants. Brincidofovir had a different mechanism of action than tecovirimat or NIOCH-14 and a relatively high barrier to development of resistance.

It was reported that brincidofovir was stored at room temperature with a shelf life of 48 months (tablet) and 30 months (oral suspension). There were plans to extend the shelf life of the formulations following review of ongoing stability monitoring data. Drug substance and drug product manufacturing processes for the tablet and suspension had been validated. Chimerix Inc. had applied for brincidofovir to become part of the United States strategic national stockpile and initial quantities would be available in the fourth quarter of 2021. Chimerix was currently reviewing regulatory strategies for the introduction of the product in other countries.

Monoclonal antibodies

Evaluate antivirals against smallpox based on monoclonal antibodies

Professor Sergei Shchelkunov shared an update on research to evaluate smallpox antivirals based on monoclonal antibodies (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 unproductive 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 the specificity of the obtained mAbs for the selected target proteins; and iv) evaluating the neutralizing properties of mAbs on vaccinia virus. As a result, cell line producers of orthopoxvirus immunodominant proteins and CHO-K1 cell lines for the production of recombinant antibodies had been developed. Three orthopoxvirus-specific human mAbs and a chimeric mAb M12B9ch specific to 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. Monoclonal antibodies will be further tested for neutralizing activity against other orthopoxviruses and variola virus. A patent for the M12B9ch antibody in the Russian Federation is expected in 2022.

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

Dr Christina Hutson also 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 the best option, mAbs and mixes of mAbs are being researched. CDC was working with several partners on this, among them Vanderbilt University, BioFactura and Macrogenics.

In 2021, CDC tested newly produced individual mAb candidates from Vanderbilt University against both variola and monkeypox viruses. Based on the results, four mixes were designed and tested against both 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.

During 2021, BioFactura sent humanized and chimeric mAbs to CDC for testing. 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. Although EC_{50} values were similar for the humanized and chimera forms of anti-B5 antibody (h8A and c8A) (0.05 and 0.09 ug/mL respectively), PRNT results showed the chimera outperformed the humanized mAb against the enveloped virion (EV) form of variola; and the anti-A33 antibodies (h6C and c6C) were found not to neutralize the EV form. The research team 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.

In summary, products from partner entities were deemed to be promising. While a concern was raised that antibody mixes had been found to have differing effects on different surrogate orthopoxviruses – highlighting the importance of testing mixes directly against variola virus – it was noted that for one mix at least there were good efficacy data against vaccinia, monkeypox and variola in vitro and against monkeypox in the non-human primate model. In completing the work, neutralization assays such as comet reduction assays, could improve understanding as to which mixes could best be used as smallpox therapeutics.

Development of a pox monoclonal antibody cocktail: research, licensing and production update

Dr Darryl Sampey provided an update on BioFactura's development of an antiviral mAb cocktail against smallpox. The project had been supported with funds from the Biomedical Advanced Research and Development Authority (BARDA). The aim had been to humanize

three antibodies, one of which was originally derived from a mouse and the other two from a chimpanzee, ultimately to determine which mAbs would be most effective against VARV.

In 2021, both chimeric and humanized cocktails had been studied. An ectromelia virus challenge study had found that chimeric and humanized mAbs were comparably potent in vivo. The formulation had been down-selected to a two-mAb combination for further development.

Next steps included work towards an Investigational New Drug (IND) application with the FDA. This would involve a series of studies, among them comprehensive in vitro neutralization studies requiring the use of live variola virus in collaboration with CDC.

Discussion on antiviral and animal model research projects

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

The Committee pointed out that licensure of two antiviral drugs (tecovirimat and brincidofovir) was an important accomplishment, and NIOCH-14 was also being well characterized in clinical studies. Thus, another of the objectives of research with live variola virus was 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 these new outcomes would need to be presented at the World Health Assembly.

In this context, the committee encouraged the WHO collaborating centres to continue to establish surrogate methods to reduce or avoid work with live variola virus wherever possible. This approach was already ongoing for heterologous expression of the F13L gene to evaluate efficacy of tecovirimat in variola variants that were not available as live virus. The use of non-variola orthopoxviruses had also proven to be effective to reduce work with live variola virus.

Participants raised several points of concern over the prospect of halting research for smallpox antivirals. Aside from potential development of resistance to tecovirimat or NIOCH-14 (which have comparable modes of action) or brincidofovir, other factors could affect their use, such as the presence of immunosuppression or interactions with other medications. Both collaborating centres provided updates on ongoing efforts to identify promising compounds. The Committee was also informed of a project to examine candidate antivirals with a different cellular target (cyclophilin A) and agreed that this approach might not be susceptible to usual mechanisms of resistance. If activity could be demonstrated against non-variola orthopoxviruses, the approach could be considered for further development for treatment of smallpox.

For effective validation of smallpox antiviral agents, an animal model had been an elusive goal. The Committee agreed that a humanized mice model which allowed variola virus infection to evolve in a manner clinically similar to natural infection in humans was superior to autologous orthopoxvirus animal models such as ectromelia in mice or rabbitpox in rabbits, which did not replicate aspects of human smallpox. While the Committee was favourable to the proposed project amendment[20] a point was made that human tissue transplanted in these mice could be studied further to reduce the use of cells from fetal tissue, which was a feature of HU-BLT mice.

[20] See Annex 3.

The Committee was in general favourable to further characterization of monoclonal antibodies as they could complement small-molecule antiviral strategies. It was acknowledged it would still take time to have a licenced product available and that the cost of any resulting product would have to be considered. Regarding dissemination of therapeutics, reference was made to discussions under way on broader intergovernmental agreement on pandemic preparedness and response. The challenges notwithstanding, passive immunization with antibodies was considered to be a promising approach, as had been seen in the COVID-19 pandemic.

In conclusion, it was agreed that the results from public health-relevant research on variola virus may also have important public health benefit for monkeypox prevention and control, as that zoonosis currently posed the most significant public health threat from orthopoxviruses. The Committee recognized that potential benefits for control of monkeypox would not have been achieved without the work to ensure preparedness for a smallpox emergency.

Recommendations made by the Committee 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 S. Shchelkunov reminded the Committee that preclinical studies in 2019 on the VACΔ6 vaccine in animals had shown high specific activity. Levels of virus-neutralizing antibodies in the blood of vaccinated rabbits, guinea pigs and mice against variola at 42 days and 6 months following vaccination had not differed significantly from those found in the blood of animals vaccinated with the commercially available live smallpox vaccine. After Phase I safety and immunogencity clinical studies had also shown no contraindications for proceeding, authorities in the Russian Federation had approved in 2020 the conduct of Phase II and III clinical studies with VACΔ6 in line with the projects approved by WHO in 2019. In 2020–2021, a double-blind, comparative, randomized, placebo-controlled study in four parallel groups had been conducted. In total, 334 volunteers aged 18–60 years had been enrolled. During the study, participants had received either a single dose of VACΔ6 vaccine (at 10^7 PFU[21]) 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. Levels of antibodies to variola virus remained stable six months post-vaccination.

Initial studies had demonstrated that both the fourth-generation VACΔ6 vaccine and classical first-generation LIVP-based[22] vaccine licenced in the Russian Federation induced generation of vaccinia virus-neutralizing antibodies in clinical trial participants. The sera of volunteers who received two doses of VACΔ6 showed no significant difference in the geometric mean antibody titres from that found in volunteers immunized with the classical

[21] Plaque-forming unit.

[22] 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.

vaccine. Approved studies began in 2021 to assess the neutralizing activity against VARV of the sera of volunteers. Work will continue in 2022 to complete the assessment for all study participants.

The main advantage of the fourth-generation VACΔ6 vaccine, compared to the commercially available classical vaccine used in the Russian Federation, was that it demonstrated lower reactogenicity while retaining immunogenic properties. Further important differences for the new vaccine were that shelf life at 2–8 °C had been extended from two years to 10 years and the new product did not contain antibiotics as preservative agents. Marketing authorization in the Russian Federation for VACΔ6 was anticipated in 2022.

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

Dr Todd Smith reported on the vaccination of health workers against monkeypox in an ongoing cohort study in Tshuapa province, in the Democratic Republic of the Congo. In this study to evaluate vaccine safety and immunogenicity in a field setting, the MVA-BN vaccine[23] 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. Participants had completed diaries to document potential exposures to monkeypox or any vaccine side effects and outcomes. The first cohort received liquid vaccine, while the second cohort were vaccinated with two doses of MVA-BN in a lyophilized formulation. No adverse events were reported. No cases of monkeypox were identified among participants during the two year follow-up. One participant from cohort 1 was diagnosed with monkeypox 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 throughout the two year study 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 representative sample of cohort 1 vaccinees against both vaccinia virus and monkeypox virus. As with the ELISA, participants were grouped according to prior smallpox vaccination status. Similar to the ELISA, participants with prior vaccination had higher and more durable virus neutralizing antibody titres. Seroconversion (a two-fold rise in titre from day 0) was similar between the two participant groups on day 42 and similar to seroconversion seen in prior BN-MVA clinical trials. However, on day 730, 51% of participants with prior vaccination had titres that remained above the threshold for seroconversion compared to 30% of participants without prior vaccination. Virus neutralization by participant sera yielded very similar results for monkeypox and vaccinia viruses. Samples from cohort 2 vaccinees will be tested by ELISA once they arrive at CDC.

Follow-up studies were planned for 2022 in the Democratic Republic of the Congo. To assess immunogenicity in a vaccine booster study and to determine if the anamnestic response remained, a third dose of MVA-BN would be administered to previous study

[23] 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.

participants. This would be five years following primary vaccination for the cohort that had received the liquid-frozen formulation, and three years for the cohort that had received the lyophilized formulation.

Research approved by WHO in 2019 and still to be undertaken included in vitro studies to evaluate the capacity of sera sampled from vaccinees long-term after MVA-BN vaccination; and LC16 vaccination (if sera are available), to neutralize IMV and the extracellular EV forms of different strains of variola virus.

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

Dr Florian Lienert provided an update on research and licensing of MVA-BN vaccine. The clinical development of the freeze-dried formulation of MVA-BN had been completed in early 2021. Ongoing studies in the Democratic Republic of the Congo were showing an excellent safety profile. A study was also under way to confirm that the shelf life of lyophilized MVA-BN would be longer at -20 °C than for the liquid frozen formulation. Bavarian Nordic was continuing the development of vaccines based on the MVA-BN vector platform, with a vaccine candidate for prevention of respiratory syncytial virus planned to enter Phase III clinical trials.

The indication for MVA-BN in Canada had been expanded in November 2020 to include prevention of smallpox, monkeypox and related orthopoxvirus infections and disease. MVA-BN was being provided for the Canadian national stockpile and new MVA-BN supply contracts were in place with three European governments. In June 2021, in response to cases of monkeypox in the United Kingdom related to travel from Nigeria, MVA-BN had been supplied upon request by the government for vaccination of health workers. It was noted that MVA-BN was generally only produced in response to large orders (typically from governments). In the United States of America in 2021, the Advisory Committee on Immunization Practices had recommended the use of MVA-BN vaccine for persons at risk of exposure to orthopoxviruses in occupational settings, including laboratory personnel, selected health workers and designated response teams.

Dr Lienert also reported the completion of a systematic review[24] to assess how monkeypox epidemiology had evolved since 1970 when the first human case had been confirmed in the Democratic Republic of the Congo. In total, human monkeypox had now appeared in 10 African countries and four countries elsewhere. The number of cases reported had increased at least 10-fold, and median age at presentation had risen from four years in the 1970s (young children) to 21 years in 2010–2019 (young adults).

Discussion on research projects related to vaccines

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

The Committee noted that the studies on the VACΔ6, MVA-BN and LC16 vaccines continued to make good progress. Antibody response in study participants in the Russian Federation (VACΔ6) and the Democratic Republic of the Congo (MVA-BN) remained

[24] Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR et al. The changing epidemiology of human monkeypox – a potential threat? A systematic review. PLoS Negl Trop Dis. 2022;16(2):e0010141. doi:10.1371/journal.pntd.0010141.

high, six months post-vaccination in the former and until day 42 in the latter. The LC16 vaccine had long been licenced in Japan. The liquid-frozen formulation of MVA-BN had been approved in Canada, the EU and the United States of America, and licensure of VACΔ6 was expected in the Russian Federation in 2022. The Committee recommended that WHO inform Member States of these vaccine approvals and that such third- and fourth-generation vaccines could be added to national vaccine stockpiles.

A number of country governments had continued to stockpile vaccines and some had requested supplies of MVA-BN for this purpose. It was noted that overall, surge capacity for orthopoxvirus vaccine production at all manufacturers may be declining and that production capacity at Bavarian Nordic was also limited to stockpile orders placed. Thus any possible emergency use of MVA-BN may at this time rely on a small number of national governments. Committee members welcomed the news that SAGE would revisit previous orthopoxvirus vaccine recommendations in 2022.

Committee members enquired about the potential for reuse of the antigen platform on which MVA-BN is based, for example for other vaccines. The representative from Bavarian Nordic indicated the company was considering the potential for further use following its development of the MVA-BN respiratory syncytial virus candidate and promising results of the human challenge trial.

The WHO collaborating centres noted that the number of reported cases of monkeypox have continued to rise. Surveillance data from the Democratic Republic of the Congo supports the hypothesis that younger age groups are contracting monkeypox in increasing numbers (now including young adults), though the reasons are as yet unclear. The Committee noted that during an eventual smallpox or monkeypox outbreak health workers would be at risk. They would therefore need to be among the first to receive vaccines to ensure they were protected during any response.

Recommendations made by the Committee 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)

As presented during the twenty-second Committee meeting, **Dr Alexander Agafonov** said the purpose of this project was to create a sensitive, rapid, easy-to-use, inexpensive, and point-of-care dot immunoassay to detect orthopoxviruses. Vaccinia virus, ectromelia, cowpox as well as other viruses such as rubella and measles were used as heterogeneous controls to test the kit. Using the prototype kit, it had been possible to detect all orthopoxviruses studied in impurified samples within the concentration range of 10^3–10^4 PFU/mL. The assay was specific and easy to use, and is planned for use in a field setting. The diagnostic kit has five arrays, which would allow for samples from five different individuals. Once the samples had been introduced to the wells, a wait time of 36 minutes was needed to determine the result.

In 2021, the kit was tested using a wide range of orthopoxvirus species (vaccinia virus, cowpox virus, rabbitpox virus and ectromelia virus), with demonstrated sensitivity of virus detection in unpurified cell culture preparations to be 10^3–10^4 PFU/mL. The kit made it possible to successfully detect viruses in clinical material such as blood, skin rashes, and organs from infected animals (rabbits and mice). The kit was also employed to detect vaccinia virus in pustules at the vaccination site in recent vaccinees. In this case, the sample used was liquid taken directly from the pustule on the skin. It was specified that the diagnostic kit had been developed for use by a trained health worker – given the sampling of infectious material, the proper method for disposal of medical waste in the field would be important.

The plan for 2022 was to test the kit using specimens or other samples containing variola or monkeypox viruses. While the end goal was use of the kit in the field, it was indicated that the kit would require additional laboratory testing before it could be field-tested and compared with other similar products. There was currently no timetable for licensure application.

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 Victoria Olson 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 both nucleic acid-based and protein-based rapid diagnostic tests. Both types of tests had advantages and disadvantages, as previously outlined. Progress had been made in adapting automated diagnostic nucleic acid amplification assays for field settings,[25] as shared during the twenty-second meeting of the Committee. Two laboratories in the Democratic Republic of the Congo were assessed for capability to conduct these assays: use of this diagnostic platform for monkeypox in remote laboratory settings appeared to be feasible, particularly as it was determined that heat inactivation of the swab prior to processing would not impair detection.

Dr Olson noted that protein-based tests provided valuable flexibility for field deployment. In this regard, evaluation of a commercial lateral flow assay (Tetracore®) for monkeypox was ongoing in the Democratic Republic of Congo, with 36 of 60 participants enrolled to date. Preliminary results had suggested that confirmation of test results in a field setting required less time (4.5 days on average) than shipping specimens to a national laboratory for PCR assay (30 days on average). However, the sensitivity of the assay compared to PCR was low (33%). CDC, in collaboration with Arizona State University, had developed another lateral flow assay technology which should provide better diagnostic sensitivity than the commercial kit discussed above. Due to the COVID-19 pandemic, however, this project is currently on hold.

In the United States of America, diagnostic development of nucleic acid amplification assays had continued over the past year 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 2021, two PCR extraction buffers[26] were shown to inactivate monkeypox virus in cell culture and tissue homogenate. Results would be submitted to the FDA for approval for use in the United States Laboratory Response

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

Network. For protein-based assays, variola-specific mAbs had been generated and optimal combinations identified, with promising detection of variola virus in a lateral flow assay format.

Discussion on research related to diagnostics

The Committee re-emphasized that diagnostic kits which could be used in the field (i.e. for point-of-care use and requiring low energy) would be critical for preparedness and response to any smallpox-like event, particularly in low-resource settings.

The Committee discussed the diagnostic kits being developed by the WHO collaborating centres. The immunoassay being developed by VECTOR required further laboratory work with monkeypox virus before it could be tested in field settings. For CDC, given the technical requirements of the real time PCR-based assay under development, it would not in the short term likely serve as a so-called "bedside" point-of-care test for use in resource-limited settings. In contrast, lateral flow assays would be useful in a wide range of settings. During discussion it was also determined that the sensitivity of the protein-based diagnostics being developed by CDC (lateral flow assay) and VECTOR (immunoassay) could not be directly compared. The Committee also noted that resources developed for smallpox preparedness are now being trialed for monkeypox interventions in the Democratic Republic of the Congo (MVA-BN, protein-based diagnostics) and the Central African Republic (tecovirimat).

The Committee then considered avenues for future work: development of orthopoxvirus diagnostics without the use of variola virus. Committee members reiterated previous recommendations that the WHO collaborating centres should enhance efforts to find ways to develop diagnostics without the use of live virus, for example, through recombinant proteins and complementary DNA. Further to the recommendations of the Committee, the World Health Assembly had endorsed that work with live virus should continue for research on antivirals. However, the Committee had on two occasions also formally recommended that live VARV was no longer needed for diagnostics or vaccine development. Any change to those recommendations should acknowledge the prior positions when stating a new recommendation. Collaborating centre presenters explained that live virus is used only at the last stage of their work following preliminary work with surrogate orthopoxviruses.

The Committee also discussed recommending a roadmap be established to leverage advances in smallpox diagnostics for the development of point-of-care diagnostics for monkeypox in low-resource settings and their deployment in a reliable and equitable manner. This would improve control strategies for monkeypox, and may also offer insight in the event of a smallpox emergency. Such a roadmap would require a clear goal and objectives so that the destination is clear, and include criteria on specificity and sensitivity of diagnostics. Reliability would be a key requirement for rapid diagnostic tests for orthopoxviruses, particularly if designed for use in the general population. Regulatory authorities should be included in discussions about target product profiles for diagnostic kits. The R&D Blueprint partners could be engaged to collaborate on the roadmap due to broad experience gained for other emerging pathogens.[27] It was noted that it would be important to discuss the rationale for the work: that is, determining if the roadmap would be specifically to address monkeypox prevention and control or also to provide proof-of-principle for detecting and responding to smallpox-like events.

[27] The R&D Blueprint is a global strategy and preparedness plan for rapid activation of research and development activities during epidemics. R&D Blueprint [website]. Geneva: World Health Organization; 2022 (https://www.who.int/teams/blueprint, accessed 23 February 2022).

While making monkeypox a priority among competing health priorities could be a challenge, it was suggested that until the current pandemic, coronaviruses also did not elicit widespread concern. Monkeypox could be a similar case and it was considered likely that the number of monkeypox cases was being underreported. Another challenge to such a roadmap could be with funding: government agencies were funding smallpox research, not monkeypox. The Rapid Acceleration of Diagnostics Initiative (RADx) of the United States National Institutes of Health,[28] established to fund research for COVID-19, is another example of a potentially useful public-private partnership; it was reported to be considering other research options, among them smallpox. It was suggested that a subgroup of the Committee could work on the topic in the coming year.

Recommendations made by the Committee on this topic can be found at the end of the report.

LESSONS LEARNED FROM THE COVID-19 PANDEMIC

The Committee undertook to discuss the implications for preparedness for smallpox-like events arising from the ongoing COVID-19 pandemic. The discussion covered a range of topics including the rapid development of vaccines and diagnostics as well as the importance of equitable deployment, the ability to synthesize severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from the sequenced genome before there was access to the virus circulating elsewhere, and lessons learned for clinical care and deployment of approved treatments. A number of observations were made and this summary also includes related comments from substantive discussions earlier in the meeting.

The Committee discussed that the COVID-19 pandemic had revealed how quickly diagnostics and vaccines could be developed and deployed when resources and political will were abundant. Notwithstanding the rapid evolution of misinformation, the pandemic had also created an awareness among the general population about public health measures and the use of diagnostic tests, vaccines and new technologies to fight emerging biological threats. The rapidity with which the world could develop nucleic acid amplification tests, such as PCR and loop-mediated isothermal amplication tests, followed by widespread development of rapid antigen tests had been due in part to the fact that the genetic sequence of SARS-CoV-2 had been shared worldwide. Innovative technologies such as messenger RNA and mRNA-carrying nanoparticles already under development had been used to rapidly develop vaccines against SARS-CoV-2. Furthermore, production capacity could be very high. The Committee agreed that new mRNA technologies may have implications for smallpox vaccines in the future. Some members advocated the further exploration of this potential as some work may already may be under way and also noted that sequence information would also be important for further development of antiviral treatments.

The Committee discussed a related lesson learned about reconstruction of SARS-CoV-2 from genetic information. In a salient example, a Committee member shared that SARS-CoV-2 DNA fragments had been received in Switzerland on 4 February 2020 and the virus chemically synthesized in a laboratory on 12 February 2020, while the first case of

[28] Rapid Acceleration of Diagnostics (RADx). RADx Programs [website]. Bethesda (MD): National Institutes of Health; 2021 (https://www.nih.gov/research-training/medical-research-initiatives/radx/radx-programs, accessed 23 Febrary 2022).

COVID-19 in the country was reported only on 24 February 2020.[29] An article discussing the virus reconstruction had been published in pre-print form online on 21 February 2020,[30] and later in the journal Nature.[31] The main point shared about this was that timely genomic sequencing and reconstruction could help in outbreak control: the information needed to develop diagnostics and vaccines could travel faster than the pandemic itself. In this example, the SARS-CoV-2 virus had been reconstructed in one week after receipt of synthetic DNA fragments based on the genetic sequence of the virus. For rapid information-sharing to become standard practice, a globally coordinated laboratory network for DNA synthesis would be required. Such benefit could be further unlocked prior to any event – this would require advancing development of mRNA vaccines for orthopoxviruses, so that the genetic information required to make vaccines could be rapidly transmitted and vaccines produced locally when needed (if the technology was also shared).

This comment was offered to further inform the discussion from day 1 about publication of all known variola virus sequences in public databases, which the Committee had previously recommended. It was noted that ultimately elimination of any virus would require removal of sequence information from all public and private databases; while most of the debate about synthetic biology technologies related to smallpox had highlighted the continuing risk of smallpox re-emergence,[32] this discussion point had illustrated that benefits could also accrue from this type of work.

Following a request from the Committee to the Secretariat about deployment of antiviral treatments for SARS-CoV-2 during the pandemic which could be used for a framework for smallpox, the Secretariat outlined some lessons learned regarding clinical care, as follows:

- clinical research could be integrated into a pandemic response as had been demonstrated by the WHO-led Solidarity Therapeutics Trial,[33]
- emerging evidence related to treatment could be regularly updated. For COVID-19, updates were shared through a living guideline format: The WHO therapeutics and COVID-19: living guideline.[34] This document contained the most up-to-date WHO recommendations for use of therapeutics in the treatment of COVID-19. This included guidance on the use of widely available corticosteroids, newly developed monoclonal antibody therapies and interleukin-6 receptor blockers, as well as guidance for specific population groups;
- the WHO prequalification and emergency use listing processes had been useful mechanisms to inform development of guidance. Prequalifying ingredients could help

[29] Swiss public health data show the first PCR-confirmed case of COVID-19 was reported on 24 February 2020. COVID-19 Switzerland [website]. Bern: Federal Office of Public Health; 2022 (https://www.covid19.admin.ch/en/epidemiologic/case?time=total&rel=abs&geo=CH, accessed 23 February 2022).

[30] Thi Nhu Thao T, Labroussaa F, Ebert N, V'kovski P, Stalder H, Portmann J et al. Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. bioRxiv. 21 February 2021 (https://www.biorxiv.org/content/10.1101/2020.02.21.959817v1.full, accessed 23 February 2022).

[31] Thi Nhu Thao T, Labroussaa F, Ebert N, V'kovski P, Stalder H, Portmann J et al. Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. Nature. 2020; 582(7813):561–65. doi:10.1038/s41586-020-2294-9.

[32] The Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox. A report to the Director-General of WHO. Geneva: World Health Organization; 2015 (https://apps.who.int/iris/bitstream/handle/10665/198357/WHO_HSE_PED_2015.1_eng.pdf;sequence=1, accessed 23 February 2022).

[33] WHO COVID-19 Solidarity Therapeutics Trial [website]. Geneva: World Health Organization; 2022 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments, accessed 23 February 2022).

[34] Therapeutics and COVID-19: living guideline, 6 July 2021. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/342368, accessed 23 February 2022).

avoid bottlenecks in supply chains – as quality-assured products could be sourced worldwide, any problem with one ingredient would become less of a challenge. Many manufacturers of products for COVID-19 had engaged with WHO early in preparation of submissions, shortening the lead time to approval and access; and

- the prompt creation of the Access to COVID Tools Accelerator mechanism had facilited access for countries to medical countermeasures including treatments and offered a mechanism to support the logistics of medical supply as needed.

In conclusion, the Committee had highlighted areas where lessons learned from the COVID-19 pandemic could be brought to bear on preparedness and development of medical countermeasures for orthopoxviruses. While there was no conclusion as to how this would affect research with live variola virus, WHO and the WHO collaborating centres were invited to take note in consideration of next steps.

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 notwithstanding delays related to the COVID-19 pandemic, there were no major departures from the agreed roadmap.

It was deemed to be too early at this time to make adjustments to the time frame of research. 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. Detailed proposals will continue to be reviewed annually. The recommendations of the Committee at the twenty-third 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 2022 *(with updates)**

Area of work	CDC	VECTOR
Genome sequencing	Complete genome sequencing of *15* isolates. *Updated to include those without epidemiological information (2022)*	Complete the genome sequencing 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 orthopoxviruses, 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	**NIOCH-14 oral formulation** Assessing the oral formulation of NIOCH-14: Complete Phase I clinical trials in 2020 Complete Phase II and III trials for 2021–2022 Complete registration in 2023–2024 **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 **Monoclonal antibodies and antibody mixes** Evaluate antivirals against smallpox 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 aged 18–40 years) by Dec 2019 Undertake Phase II and III clinical trials in 2020–2021 and assess variola virus neutralizing antibody titres from sera of participants Complete registration in 2022
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 *Proposal amended to include a Hu-BLT efficacy study or CD34 follow-up study*	

* *Edits in italics are new from November 2021 compared to the plan set out in 2019–2020.*
Note: This multi-year research agenda was presented to the WHO Advisory Committee on Variola Virus Research at their 21st, 22nd and 23rd meetings in individual presentations. Proposals were reviewed by Committee members in order to provide recommendations to WHO as outlined further in this report.

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.

The Committee pointed out that licensure of two antiviral drugs (tecovirimat and brincidofovir) was an important accomplishment, which was a need discussed by the Seventy-second World Health Assembly. Similarly, the Committee noted the promising work related to third- and fourth-generation vaccines and expressed concern about the limited production capacity of licenced orthopoxvirus vaccines; the Committee noted that WHO could inform its Member States of recent licensures of third-generation vaccines, which could be added to national stockpiles. In discussions of diagnostics, the Committee noted the work done by the WHO collaborating centres, which has led to promising developments for point-of-care diagnostic tests for smallpox. In this field work, monkeypox has been used as a surrogate for variola virus and the Committee noted that resources developed for smallpox preparedness will be vital to control a continued or accelerated emergence of monkeypox – as that zoonosis currently posed the most significant public health threat from orthopoxviruses – and that this is a major immediate potential benefit of the research that takes place using variola virus under the oversight of WHO.

The following section summarizes the recommendations of the Committee as articulated at its twenty-third meeting. Text in parentheses indicates to whom each recommendation is directed.

General

- Complete sequencing of variola virus strains 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 and 2020 but delayed by the COVID-19 pandemic (WHO collaborating centres). The committee further recommended approval of the project amendment to evaluate the humanized mouse model.
- Ensure that the investment in variola virus research for smallpox preparedness is fully leveraged to offer solutions to monkeypox outbreaks in endemic countries (WHO and WHO collaborating centres).
- Continue to leverage lessons learned from the ongoing COVID-19 pandemic in considering avenues for smallpox preparedness and control of diseases linked to emerging orthopoxviruses (WHO and WHO collaborating centres).

Antivirals

- Aim to complete the direct full genome sequencing of all remaining variola virus strains or isolates, including those without epidemiological data, within two years, in order to identify genetic markers of resistance to antivirals (WHO collaborating centres).
- Continue to establish surrogate methods to reduce or avoid work with live variola virus wherever possible (WHO collaborating centres).
- Pursue further characterization of monoclonal antibodies as they could complement small-molecule antiviral strategies (WHO collaborating centres).
- Ensure parallel development for monkeypox treatment in smallpox antiviral development projects (WHO collaborating centres and WHO).

Vaccines

- Inform Member States that third-generation vaccines (LC16 and MVA-BN) are licenced in some jurisdictions and could be added to national vaccine stockpiles (WHO).
- Continue efforts to characterize the effectiveness against other orthopoxviruses of smallpox vaccines approved or in development, and support studies particularly against monkeypox in field settings (WHO collaborating centres and WHO).

Diagnostics

- Establish a roadmap to leverage advances in smallpox diagnostics for further development of point-of-care diagnostics for monkeypox (WHO).
- Involve regulatory authorities in development of target product profiles for orthopoxvirus diagnostics (WHO).
- Continue to work towards development of orthopoxvirus diagnostics with additional focus on approaches that do not require recourse to the use of live variola virus (WHO collaborating centres).
- Expedite the availability of (rapid, point-of-care) orthopoxvirus diagnostic tests in the field in a reliable and equitable manner , including the considerations discussed at this meeting (WHO collaborating centres and WHO).

ANNEXES

ANNEX 1 | AGENDA

Agenda

Objectives of the meeting

- review progress of approved research with live variola virus; and
- update research programme and recommendations for 2020-2022.

Wednesday, 3 November 2021 – Day one – Chair: Andreas Nitsche

Session 1. Opening and report of the WHO Smallpox Secretariat		
Time		
13:00	Opening remarks	Dr S. Briand, Director, Health Emergencies Dr M. Van Kerkhove, Head, Emerging Diseases and Zoonoses Unit
13:15	Roll call, declarations of interest Report of the Smallpox Secretariat	Dr R. Lewis WHO Smallpox Secretariat
14:00	Discussion	*All*
Session 2. WHO Collaborating Centre reports - Variola virus and DNA collections		
14:10	Summary of proposals for research with live variola virus	Chair ACVVR
14:15	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	Dr R. Maksyutov, Director General, FBRI SRC VB VECTOR, Rospotrebnadzor, Russian Federation
14:25	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 V. Olson, Chief, Poxvirus and Rabies Branch, Centres for Disease Control, Atlanta, Georgia, USA
14:35	**Discussion**	*All*

Session 3. ANTIVIRALS - *progress reports*		
14:45	Clinical trials on the anti-smallpox drug NIOCH-14; and Discover and test novel chemical antivirals for smallpox treatment and prevention (Approved 2019)	Dr A. Agafonov, Deputy Director General for Research, VECTOR
14:55	Discussion on VECTOR antivirals research projects	*All*
15:10	Use of live variola virus to: - characterize effectiveness of antiviral therapeutic tecovirimat (additional data, Approved 2019); - characterize effectiveness of novel antiviral therapeutic ST-357 (Approved 2019); and - determine whether mice are a suitable animal model for human smallpox (Partially approved 2019; Approved 2020).	Dr T Smith, CDC Dr C Hutson, CDC
15:25	New perspectives: restriction of orthopoxviruses by TRIM5α	Professor G Smith, Cambridge University
15:35	Tecovirimat and ST-357 – licensing & production update	Dr D Hruby, SIGA Technologies
15:45	Brincidofovir – licensing & production update	Dr O Naderer, Chimerix
15:55	Discussion on CDC antivirals research projects	*All*
16:10	Break 10 minutes	

Session 4. MONOCLONAL ANTIBODIES - *progress reports*		
16:20	Evaluate antivirals against smallpox based on monoclonal antibodies (Approved 2019); *and* Replenishment of stocks with non-infectious material, derived from live variola virus, required for diagnostics development Approved 2020	Professor S Shchelkunov VECTOR
16:30	Use of live variola virus to evaluate antivirals (monoclonal biologics) against variola virus (Approved 2019)	Dr C Hutson CDC
16:40	Development of a pox monoclonal antibody cocktail – research, licensing & production update	Dr D Sampey Biofactura
16:50	**Discussion** on monoclonal antibody research projects	*All*
17:00	Close of day one	

Thursday, 4 November 2021 – Day two – Chair: David Ulaeto

Session 5. VACCINES - *progress reports*		
13:00	Recap of discussions and recommendations of day 1	Chair
13:20	Clinical trials of the anti-smallpox vaccine VACdelta6 and Assess the neutralizing activity of vaccinated volunteers' sera and the sera of vaccinated animals to support development of 4th generation smallpox vaccine (Approved 2019)	Prof S Shchelkunov VECTOR
13:40	Discussion on VECTOR vaccine research	All
13:50	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
14:00	MVA-BN vaccinia vaccine – research, licensing and production update	Dr F. Lienert Bavarian Nordic
14:10	**Discussion** on CDC vaccine research updates and plans	*All*

Session 6. DIAGNOSTICS - *progress reports*		
14:20	Develop advanced methods for rapid point-of-care diagnostics of orthopoxvirus infections. (Approved 2020)	Dr A Agafonov, Deputy Director General for Research, VECTOR
14:30	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 V Olson CDC
14:40	**Discussion** of diagnostic research updates, plans, next steps *including* **Roadmap for diagnostic development without use of variola virus**	*All*
15:00	**Discussion** on live variola virus research agenda 2020–2022, recommendations and pending issues	*All*
Session 6. Lessions learned from the pandemic – open discussion		
15:15	Lessons learned from COVID-19 - implications for smallpox/orthopoxvirus preparedness and Medical Countermeasures development	*All* Discussion led by Chair
16:00	Break 10 minutes	

Session 7. Closed session		
16:15	Administrative and technical matters, recommendations	*All*
17:00	Close of day two and 23rd ACVVR	

ANNEX 2 | PARTICIPANTS[35]

CO-CHAIRS

Dr Andreas Nitsche (Chair, day 1), Head of Division, Highly Pathogenic Viruses Centre for Biological Safety, Robert Koch Institute, Berlin, Germany

Dr David Ulaeto (Chair, day 2), Principal Scientist, Department of Biomedical Sciences, Defence Science and Technology Laboratory (DSTL), Salisbury, United Kingdom

MEMBERS

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

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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 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

Professor Geoffrey L. Smith, Head, Department of Pathology, University of Cambridge, Cambridge, United Kingdom

[35] In alphabetical order by section.

Professor Muyembe Tamfum, Director, Institut National de Recherche Bio-Médicale (INRB), Kinshasa, Democratic Republic of the Congo

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

Professor Henda Triki*, Chief, Laboratory of Clinical Virology, Institut Pasteur de Tunis, Tunis Belvédère, Tunisia

Dr Zalini binti Yunus*, Senior Director, Biological & Toxin Weapons Convention Nucleus, Science & Technology Research Institute for Defence (STRIDE), Ministry of Defence, Kajang, Malaysia

INVITED OBSERVERS (Temporary Advisors)

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

Dr Clarissa Damaso, Associate Professor, Instituto de Biofisica Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

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

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

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

INVITED PRESENTERS

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

Dr Elena V. Gavrilova*, Deputy Director General for Research, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor, Russian Federation

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

Dr Christina L. Hutson, Lead, Virus-Host Molecular Interactions Team (VHMI), Poxvirus and Rabies Branch (PRB), 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

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

Dr Odin Naderer, Vice President, Clinical Pharmacology/Translational Medicine Chimerix, Durham, North Carolina, United States of America

Dr Victoria Olson, 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, Georgia, United States of America

* Unable to attend

Dr Darryl Sampey, Biofactura, President and CEO, Ste. Z Frederick, 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

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

* Unable to attend

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

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Ms Alexandra Hill, Technical Officer, WHE/IHM/IEP

Dr Kazunobu Kojima, Technical Officer, WHE/CPI/PCB

Mr Kai Lashley, Rapporteur

Dr Rosamund Lewis, Head of Smallpox Secretariat, WHE/IHM/PAT/EZD

Ms Anne Menthon, Assistant to Unit Head, WHE/IHM/PAT/EZD

Dr Mike Ryan*, Executive Director, WHE

Dr Maria Van Kerkhove, Unit Head, WHE/IHM/PAT/EZD

* Unable to attend

ANNEX 3 | RESEARCH PROPOSALS

Annex 3a. Research proposals presented for 2022 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*	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 and 2021 due to the COVID-19 pandemic. There was consensus in the Committee to endorse continuation of privously approved projects.

Annex 3b. Research proposals presented for 2022 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 [continuing]				Continuation*	December 2019
5. Determine whether mice are a suitable animal model for human smallpox, providing means to evaluate medical countermeasures against authentic agent [continuing]				Continuation*	November 2020
5bis. Determine whether mice are a suitable animal model for human smallpox, providing means to evaluate medical countermeasures against authentic agent [request for extension/amendment]	7	0	Yes	Amendment requested at 23rd ACVVR meeting. Proposal circulated after the meeting. Approved by WHO	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. There was consensus in the Committee to endorse continuation of privously 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

Maksyutov RA, Shchelkunov SN
WHO Collaborating Centre 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

Organization of and experimentation with the Russian variola virus (VARV) collection at the World Health Organization (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 (standard operating procedures) have been developed that govern the implementation of research and 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 have been established.

Currently, the VARV collection comprises 120 strains, originating from Europe, Asia, Africa, South America and the Middle East. The VARV strains in the repository are stored in a freeze-dried or frozen form or in the form of primary specimens (from scabs) isolated from human patients in the past. As reported at the twenty-third WHO Advisory Committee on Variola Virus Research meeting in November 2021, there remain 88 variola virus strains to be fully sequenced.

The work with live VARV was resumed in 2021 following the WHO approval of the projects proposed in November 2020. Only one study involving the use of live VARV has been conducted this year: to evaluate the neutralizing activity of sera of vaccinated volunteers and those vaccinated animals using live VARV to support the development of less reactogenic fourth-generation smallpox vaccines.

The work with live VARV is scheduled to be resumed in early 2022 following the WHO approval of the VECTOR-proposed research projects involving the use of live VARV.

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

Assessment of the neutralizing activity of vaccinated volunteers' sera and those of vaccinated animals using VARV to support the development of less reactogenic fourth-generation smallpox vaccines.
Discovery and testing of novel chemical antivirals for smallpox treatment and prevention.
Use of live VARV to evaluate antivirals against smallpox based on monoclonal antibodies.

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

The progress detailed above and plans for 2022 were reported at the 23rd WHO ACVVR meeting in November 2021.

Clinical trials on the anti-smallpox drug NIOCH-14

Agafonov AP, Shishkina LN, Mazurkov OY, Usova SV, Bogryantseva MP, Ginko ZI, Orlovsky VG, Kuzubov VI, Maksyutov RA
WHO Collaborating Centre 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

Clinical trials were conducted of the finished dosage form (FDF) of the anti-smallpox drug NIOCH-14 formulated as capsules (200 mg) for oral administration, in accordance with the Protocol No. NIOCH-01/20 titled "Open-label randomized study of the safety, tolerability, pharmacokinetics of the drug NIOCH-14 in volunteers aged 18-50 years in parallel groups".

The study involved six groups of volunteers: group 1 included 15 volunteers who received a single oral dose of NIOCH-14 (200 mg); group 2 included 15 volunteers who received a single oral dose of NIOCH-14 (600 mg); group 3 included 15 volunteers who received a single oral dose of NIOCH-14 (1200 mg); group 4 included 15 volunteers who received 200 mg orally daily for 6 days; group 5 included 15 volunteers who received 600 mg orally daily for 6 days; group 6 included 15 volunteers who received 1200 mg orally daily (600 mg, twice a day) for 6 days.

Regardless of single or multiple dose administration of the drug (200, 600 or 1200 mg), no changes were reported in the blood of the volunteers (numbers of erythrocytes, leukocytes, platelets; differential leukocyte count, haemoglobin levels and erythrocyte sedimentation rate). Furthermore, no changes were reported in the levels of alanine transaminase (ALT), aspartate transaminase (ALT), alkaline phosphatase, total protein, total bilirubin, glucose, creatinine, urea, thymol test, C-reactive protein or the prothrombin index. There were no changes in urinalysis indicators during the follow-up period. No adverse events were reported with any single or administration protocol of the drug nor with multiple administration of 200 mg and 600 mg doses. With a daily dose of 1200 mg, one volunteer reported pain in the right hypochondrium and in the anticardium on the second and fifth days; this volunteer had no changes in AST or ALT (reflecting the functional status of the liver).

When studying the pharmacokinetics of the drug, it was found that ST-246[36] is an active metabolite of the chemical compound NIOCH-14.[37] The study of the main pharmacokinetic parameters of ST-246 was performed by studying the blood plasma of the volunteers in a sampling of 6–7 people from each of the six above-mentioned groups. This was done in order to assess parameters of bioavailability in single and multiple administrations of NIOCH-14 in doses of 200, 600 and 1200 mg.

[36] 4-trifluoromethyl-N- (3,3a, 4,4a, 5,5a, 6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop [f] isoindole-2 (1H) -yl) -benzamide.
[37] 7- [N`- (4-trifluoromethylbenzoyl) -hydrazinocarbonyl] -tricyclo [3.2.2.02.4] non-8-en-6-carboxylic acid.

The maximum concentration of ST-246 in blood plasma (C_{max}) for each group is shown below. Group 1 reached 215 ng/mL after 6 hours (T_{max}) and the half-life (T1/2) is 17.8 hours; group 2: C_{max} = 375 ng/mL, T_{max} = 6 hours, $T_{1/2}$ = 19.8 hours; group 3: C_{max} = 1212 ng/mL, T_{max} = 6 hours, $T_{1/2}$ = 29 hours; group 4: C_{max} = 189 ng/mL, T_{max} = 6 hours, T1/2 = 26 hours; group 5, C_{max} = 740 ng/mL, T_{max} = 6 hours, $T_{1/2}$ = 29 hours; and group 6: C_{max} = 607 ng/mL, T_{max} = 96 hours, $T_{1/2}$ = 18 hours.

The data obtained demonstrate the FDF formulation of the anti-smallpox drug NIOCH-14 to be safe and bioavailable when administered once or twice orally per day to volunteers in the form of hard gelatine capsules containing 200 mg of NIOCH-14 and excipients.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third WHO Advisory Committee on Variola Virus Research meeting in November 2021.

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

Agafonov AP, Shishkina LN, Bormotov NI, Serova OA, Mazurkov OY, Protsenko MA, Maksyutov RA

WHO Collaborating Centre 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 discovery of novel antivirals for treatment and prevention of smallpox remains relevant.

In 2019–2021, at FBRI SRC VB VECTOR, Rospotrebnadzor, more than 400 novel compounds belonging to different classes of chemical compounds were tested in vitro in surrogate orthopoxviruses (vaccinia, cowpox and ectromelia viruses). Chemical compounds containing fragments of monoterpenes, camphor, borneol, or adamantane were created at N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry (NIOCH) of the Siberian Branch of the Russian Academy of Sciences (SB RAS); benzoyl-containing adamantane derivatives were synthesized at Samara State Technical University (SamSTU); and derivatives of hydroxyimidazoles containing ester or amide fragments were provided by D.I. Mendeleev University of Chemical Technology of Russia (D.I. Mendeleev RCTU).

It was found that nine compounds containing fragments of monoterpenes or adamantane synthesized at NIOCH, SB RAS, exhibited antiviral activity against vaccinia virus with a selectivity index (SI) greater than 376. Three of these nine compounds, based on isobornylamine with a bicyclic scaffold and phenylamide or benzylamide fragments, had SI values from 2322 to 7343. Of the chemical compounds designed at SamSTU, eight had SI values of greater than 60 while three related to N-adamantylated aryl carboxamides had SI values for vaccinia virus varying from 1090 to 2320.

In addition, 26 compounds containing a monoterpene scaffold, fragments of camphor, borneol or adamantane, provided by NIOCH, SB RAS, showed activity against surrogate orthopoxviruses with SI values ranging from 85 to 18 166. At the same time, the SI values of derivatives of hydroxyimidazoles containing ester fragments or amide fragments synthesized at D.I. Mendeleev RCTU were in the range from 102 to 1152.

Thus, in further testing using live variola virus (VARV), it is proposed to include at least 25 chemical compounds with the highest SI values for surrogate orthopoxviruses. Therefore, it is necessary to continue research in order to discover chemical compounds of different classes that would be promising for the development of drugs against orthopoxviruses pathogenic for humans and animals, including VARV.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third WHO Advisory Committee on Variola Virus Research meeting in November 2021.

Evaluation of antivirals against smallpox based on monoclonal antibodies

Merkuleva YuA, Shanshin DV, Shcherbakov DN
WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at Federal Budgetary Research Institution – State Research Center of Virology and Biotechnology, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk region, 630559, Russian Federation

Three orthopoxvirus-specific human monoclonal antibodies were produced at FBRI SRC VB VECTOR, Rospotrebnadzor: A31, B23, H72 targeting A27, B5 and H3 proteins and chimeric monoclonal antibody M12B9ch specific to the L1 protein.

To produce monoclonal antibodies in the scFv-Fc format in mammalian cells pVEAL integrative plasmid vector utilizing Sleeping Beauty transposon technology was developed. VH and VL domain coding sequences of immunoglobulins were joined by GS-linker sequence and cloned to pVEAL in frame with V19 signal peptide and human IgG1 CH2-CH3 domains, which provided dimerization of two polyproteins to form an antibody in mammalian cells. Antibody stable-producing strains were obtained using the Chinese hamster ovary (CHO-K1) cell line and cultured on roller bottles. Recombinant antibodies were then purified by protein A affinity chromatography.

Orthopoxvirus recombinant antigens were obtained using CHO-K1 cells. Extra domain-coding regions of A30, B7, M1 proteins (orthologs of A27, B5 and L1) were amplified from a plasmid containing a fragment of the variola virus India-1967 strain genome by polymerase chain reaction (PCR). In addition, H3 and D8 protein sequences of vaccinia virus, variola virus, cowpox and monkeypox viruses were aligned and consensus sequences were synthesized and cloned in the pVEAL2 vector. Proteins produced in stable expression were then chromatographically purified.

The specificity of recombinant A31, B23, H72 and M12B9ch scFv-Fc monoclonal antibodies was measured by dot-blot analysis and enzyme-linked immunosorbent assay (ELISA) with the use of vaccinia virus LIVP strain lysate and recombinant orthopoxvirus antigens. Dot-blot analysis and ELISA showed the specific interaction of produced antibodies with their targets.

Vaccinia virus LIVP strain plaque reduction neutralization test (PRNT50) was used to assess the neutralizing activity of monoclonal antibodies in vitro. According to the results of the experiment, all three human monoclonal antibodies neutralized the vaccinia virus in the absence of complement 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. Next, M12B9ch

antibody was evaluated in a PRNT50 test in the presence of 2.5% of human complement and showed neutralizing activity at a concentration of 0.002 µg/mL.

Monoclonal antibodies will be further tested for neutralizing activity against variola virus and other orthopoxviruses. The patent for the M12B9ch antibody in the Russian Federation is expected in 2022.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third WHO Advisory Committee on Variola Virus Research meeting in November 2021.

Clinical trials of the VACΔ6 vaccine against smallpox and other orthopoxvirus infections

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A VACΔ6 cell-derived vaccine based on the LIVP strain of vaccinia virus with six inactivated virulence genes was developed at FBRI SRC VB VECTOR, Rospotrebnadzor.
The inactivation of the selected virulence genes was demonstrated not to affect the reproductive properties of the virus in mammalian cell cultures such as CV-1, Vero and 4647. The VACΔ6 strain is characterized by a significantly lower reactogenicity and neurovirulence compared to the original clonal LIVP variant; it induces generation of significantly higher levels of virus neutralizing antibodies compared to the original clonal LIVP variant, and provides full protection for laboratory animals in protection experiments.

The vaccine against smallpox and other orthopoxvirus infections is a lyophilized product that contains a live cell-derived VACΔ6 vaccine grown in a continuous cell line 4647. A finished dosage formulation of VACΔ6 vaccine was developed and vaccine batches produced to conduct preclinical studies on specific activity and safety in various animal models. In all studies, the first-generation smallpox LIVP-based vaccine currently approved for use in the Russian Federation was used as a reference vaccine. This vaccine may cause well-described adverse reactions known to be associated with the use of the first-generation vaccines.

The preclinical studies on the VACΔ6 vaccine in various animal models have shown high specific activity. The levels of virus-neutralizing antibodies in the blood of vaccinated rabbits, guinea pigs and mice against variola virus were determined (at 42 days and six months following vaccination). The antibody levels did not differ significantly from those found in the blood of animals vaccinated with the commercially available live smallpox vaccine used in the Russian Federation for vaccination.

The preclinical safety study of VACΔ6 vaccine was carried out in accordance with the *Guidelines for conducting preclinical studies on drugs*[38]. Based on the results of the preclinical studies, a conclusion about the absence of contraindications for proceeding with the clinical studies was received.

Following the preclinical studies, in February 2019 the ministry of health of the Russian Federation issued an authorization to conduct Phase I clinical trials. In May 2019 an open-label controlled clinical study began on the safety and tolerability of the live cell-derived vaccinia-based vaccine (VACΔ6) against smallpox and other orthopoxvirus infections, in volunteers aged 18–40 years. Authorization of the ministry of health of the Russian Federation No. 380 dated 30 July 2020 was then granted to conduct Phase II and Phase III clinical studies on the smallpox VACΔ6 vaccine in 334 volunteers. These clinical studies were completed in September 2021.

The main advantage of the investigational vaccine, VACΔ6, compared to the commercially available first-generation smallpox vaccine used in the Russian Federation, is its lower reactogenicity with retained immunogenic properties. Marketing authorization for VACΔ6 in the Russian Federation is anticipated in 2022.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third WHO Advisory Committee on Variola Virus Research meeting in November 2021.

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

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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 20–40 °C.

The evaluation of the effectiveness of detection of vaccinia virus (strain LIVP, genetically modified strains ABCNJ and A34R_[D110N_K151E]), cowpox virus (strain GRI-90), rabbitpox virus (strain Utrecht), and ectromelia virus (strain K-1) has demonstrated the sensitivity of virus detection in unpurified cell culture preparations to be 10^3–10^4 PFU39/mL. The test does not detect any cross-reactions with heterogeneous viruses (measles, rubella, and varicella) that cause exanthematous diseases.

[38] Mulyar AG, Chichenkov ON, editors. Guidelines for conducting preclinical studies on drugs. Moscow: Grif and K; 2012.
[39] Plaque-forming units.

The kit makes it possible to successfully detect viruses in clinical material from infected animals (rabbits and mice) such as blood, skin rash lesions, organs; it also allows for the detection of vaccinia virus in the scabs of pustules at the vaccination site in patients. This kit remains stable at 4–8 °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 outside of laboratories. Equipment has been built to scale up production of this kit.

Future work includes testing the kit using variola and monkeypox viruses pathogenic for humans.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third WHO Advisory Committee on Variola Virus Research meeting in November 2021.

Abstracts from CDC

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

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The World Health Organization (WHO) Collaborating Centre for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention (CDC) 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 smallpox eradication programme. Currently, the variola virus collection at CDC comprises 360 non-identical isolates. The virus collection is maintained in two separate freezers, one of which is a back-up freezer remaining largely untouched. In the United States of America, *Variola virus* is a select agent and is subject to the select agent regulations (42 CFR part 73).

Secure databases addressing WHO recommendations and United States Federal Select Agent Program requirements have been constructed and maintained to track usage of variola virus. Enhancing our database of variola virus sequences and in-silico analysis capabilities will improve our understanding of how to best use medical countermeasures such as diagnostics and antiviral therapies. 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 2021.

In 2020, 20 original variola virus specimens were processed and sequenced without propagation. The compilation of variola virus samples sequenced in previous years has been completed

In 2021, sample analysis of original isolates removed from the repository freezer in 2020 was conducted. These additional isolates were selected based on historical information available such as geographic location, date, and epidemiologic data.

Since the twenty-second meeting of the WHO Advisory Committee on Variola Virus Research held in November 2020, WHO-approved research activities using variola virus from the repository have focused on: 1) regeneration of non-infectious material for diagnostic support; 2) evaluation of monoclonal antivirals (monoclonal biologics); and 3) determining whether humanized mice are a suitable animal model for human smallpox.

The laboratory space was in active use from 24 July 2020 to 12 April 2021. The laboratory underwent decontamination prior to preventive maintenance in April 2021 and remained inactive (“cold”) for a period due to the pandemic. The laboratory became active (“hot”) again on 20 July 2021. During August 2021, the Federal Select Agent Program conducted an inspection and had no specific recommendations.

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

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

The United States Food and Drug Administration (FDA) approved the therapeutic agent tecovirimat[40] in 2018. It has been tested extensively in vivo with multiple animal models, including the non-human primate variola virus (VARV) model. In the United States of America, VARV is subject to the Select Agent Regulations (42 C.F.R. part 73). Although tecovirimat has shown efficacy against several different orthopoxviruses, it has also been noted that changes in the orthopoxviral F13L gene allow for resistance to tecovirimat; additionally, resistance emerged during use of the drug for an extended course of treatment in an individual with progressive vaccinia. Therefore, the FDA requested a post-marketing requirement/commitment in regard to testing antiviral activity of tecovirimat against an expanded panel of VARV isolates.

The objective of this study was to screen representative VARV isolates from different F13L amino acid variants in vitro against tecovirimat. Using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information, 53 published F13L genes were identified from GenBank. These, along with unpublished genomes recently generated by CDC (total n=215), were compared by visual examination of multi-sequence alignment and haplotype analyses. Eighteen nucleotide haplotypes (unique sequences) were identified, which translated to 10 unique amino acid variants, with unique variants identified only within the new unpublished CDC sequence data. In the CDC repository, isolates from six of the 10 variants were available for screening. Variant 1 reflects 78.1% of all isolates (94.7% of all isolates belong to one of three amino acid variants (1, 5, or 7)). Variants 3, 4, 6, 8, 9 and 10 represent only one isolate each. Multiple isolates from variant 1 and 5 were tested previously. Variant 6 was identified from an isolate only available at the State Research Center of Virology and Biotechnology (VECTOR) repository, which has also been previously tested with a compound of similar structure to tecovirimat. Variant 8, 9 and 10 were identified from unpublished sequences at CDC; however, samples were unavailable for further testing (e.g. they had been used to completion for sequencing).

Using a cytopathic effect (CPE) assay as described at the twenty-first Advisory Committee on Variola Virus Research (ACVVR) meeting, seven VARV isolates from five amino acid variants were screened for sensitivity to tecovirimat. Results showed all isolates were sensitive with half-maximal effective concentrations (EC_{50}) of 0.01–0.03 μM. The 90% effective concentration (EC_{90}) was also in the nanomolar range (0.02–0.15 μM) for all isolates. These results increase confidence in the effectiveness of tecovirimat as a medical

[40] Commercialized as TPOXX®.

countermeasure and help to meet the post-marketing commitment with FDA. As CDC finalizes genome sequencing of available VARV isolates and we complete our genetic database, additional variants may be identified. For isolates that are unavailable for tecovirimat sensitivity testing, we employ the strategy of expressing the unavailable F13L amino acid variants in stable cell lines. Tecovirimat sensitivity will be evaluated using CPE in a complementation assay by infecting the stable cell lines with a vaccinia virus IHD-J with F13L deleted (approved by CDC Institutional Biosafety Committee: 2019.394). This approach avoids creating a recombinant virus that contains VARV sequences. As detailed at the twenty-second ACVVR meeting (2020), we have obtained approval to generate stable cell lines expressing different VARV F13L protein variants. Cloning and site-directed mutagenesis have been completed in 2021.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third meeting of the Advisory Committee on Variola Virus Research (ACVVR) in 2021.

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

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

The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox re-emerges and this includes the development of antiviral treatment strategies. Recently the United States Food and Drug Administration (FDA) granted licensure for smallpox treatment to the antivirals tecovirimat and brincidofovir.[41] However, concerns remain regarding potential limitations of these therapies and pivotal clinical trials cannot be conducted in humans as the disease has been eradicated. As recently demonstrated in a clinical trial for Ebola virus disease, even therapeutics that are highly effective in the non-human primate model can fail in human clinical trials. Furthermore, having a range of medical countermeasures against pathogens with pandemic potential helps ensure options exist, should one treatment fail. Currently, another antiviral compound (ST-357 or TTP-018) has been identified as targeting the viral mRNA poly-A polymerase encoded by E1L, which is a different target from that of tecovirimat and brincidofovir.

During the first year of approved research for development of ST-357 as an antiviral agent for smallpox (2019–2020), the parent compound ST-357 was screened against three VARV isolates and two ST-357 analogues were screened against vaccinia virus strain Western Reserve. The three VARV isolates were: VARV Bangladesh_1974_Solaiman; Sierra Leone_1969_V68-258; and Japan_1951_Harper. All three isolates were sensitive to ST-357 with half-maximal effective concentrations (EC_{50}) of 0.04–0.05 µM. The two ST-357 analogues showed EC_{50} of 0.14–0.31 µM against vaccinia virus compared to

[41] Products commercialized as TPOXX® (tecovirimat) and Tembexa® (brincidofovir) respectively.

0.055 µM for the parent compound. Due to funding delays for identifying additional ST-357 analogues and due to the coronavirus disease 2019 (COVID-19) pandemic, we have been unable to perform additional testing in 2021.

VARV is a select agent and is subject to the select agent regulations (42 CFR part 73).

The progress detailed above under this proposal and plans for 2022 were reported at the 23rd Advisory Committee on Variola Virus Research (ACVVR) meeting in November 2021.

Evaluation of monoclonal biologics against live variola virus

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

The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox re-emerges. For therapeutics available to date, it was shown that single amino acid changes in orthopoxviruses can lead to resistance to tecovirimat, and there are safety concerns for use of brincidofovir. Multi-therapeutics with different targets may provide the best protection overall. During the coronavirus disease 2019 (COVID-19) pandemic, monoclonal antibody (mAb)-based therapeutics received Emergency Use Authorization for treatment of COVID-19. For orthopoxviruses, a mAb mix would include at least two mAbs to target both infectious forms of the virus (extracellular enveloped virions (EV) and intracellular mature virions (IMV)) to reduce the likelihood of viral resistance.

Previously, we tested 50 mAbs developed by Vanderbilt University against VARV EV and IMV in plaque reduction neutralization (PRNT) assays in the absence or presence of 10% complement. Of these, three anti-L1 mAbs neutralized more than 50% of the plaques against VARV IMV in the absence of complement. One of those mAbs is present in mAb cocktails (Mix 4 and Mix 6) that demonstrated protection in vivo against vaccinia virus. Seven mAbs neutralized more than 50% of the plaques against VARV EV in the presence of complement. Effective concentration $(EC)_{50}$ values for those mAbs and for Mix 4 and Mix 6 were determined. Mix 4 and Mix 6 had moderate neutralizing activity and included anti-B5 and anti-A33 mAbs that neutralized VARV at 50% or below. None of the VARV EV potent neutralizing mAbs are present in Mix 4 or Mix 6 (selected before VARV neutralization testing). Although treatment with Mix 4 provided protection against monkeypox virus (MPXV) challenge in the prairie dog (80% survival), there were moderate clinical signs of infection and no detectable EV neutralization in serum prior to ~12 days post-infection (manuscript in preparation). We confirmed specific mAbs' ability to neutralize the EV form of MPXV to compare with published data and identify optimal mAbs for a therapeutic compound. In 2019, we selected promising neutralizing mAbs (two for IMV, two for EV) that neutralize both VARV and MPXV at 50% or below. In 2019–2020, Vanderbilt University began producing the mAbs in Chinese hamster ovary (CHO) cells (a method used for other FDA-approved countermeasures). In 2021, we completed testing of the newly produced individual mAb candidates against VARV and MPXV. Based on those data, four mixes were made (Mix 2, Mix 2*, Mix 3, and Mix 4), and testing against both IMV and EV for VARV and MPXV was completed. All mixes appeared

to be effective against IMV and EV forms of both VARV and MPXV with IC_{50} values between 0.05–1.9 µg/mL.

Commercial entities are also producing mAbs. The Biomedical Advanced Research and Development Authority (BARDA) awarded a contract to BioFactura for its Smallpox Biodefense Therapeutic. CDC has continued to evaluate BioFactura mAbs as they are optimized, determining EC_{50} values against both the IMV and EV forms of VARV for humanized variants for each of three potential mAb drug product components [anti-VACV L1 (7D11), anti-VACV B5 (8A) and anti-VACV A33 (6C)]. The anti-L1 humanized antibody (h7D11) was found to be non-inferior to chimeric antibody (c7D11) in neutralizing the IMV form of VARV by PRNT with EC_{50} concentrations of <0.01 µg/mL, with or without complement. While EC_{50} values were similar for humanized (h8A) and chimera (c8A) forms of anti-B5 antibody (at 0.09 and 0.05 µg/mL, respectively), the chimera outperformed the humanized mAb against the EV form of VARV by PRNT. The anti-A33 antibodies (h6C and c6C) did not neutralize VARV EV. It was concluded that larger scale production should continue for both 7D11 and 8A mAbs, with in vitro neutralization tests against VARV at critical production steps.

In the USA, variola virus (VARV) is subject to Select Agent Regulations (42 C.F.R. part 73). The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third meeting of the Advisory Committee on Variola Virus Research (ACVVR) in 2021.

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

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In the USA, variola virus (VARV) is subject to the Select Agent Regulations (42 C.F.R. part 73). Historically, laboratory research efforts have tested several animal species for susceptibility to VARV. To date, non-human primates (NHPs) are the only non-human animals which exhibit overt illness. Limitations of the NHP and other surrogate orthopoxvirus models, such as short disease incubation periods which do not resemble human smallpox, mean these systems are suboptimal for evaluating efficacy of antiviral therapeutics. Humanized mice offer a possible alternative to NHPs for modelling human disease and investigating human-specific therapeutic candidates.

Previously, we published results showing three types of humanized female mice (BLT, hu-CD34 and PBMC), obtained from Jackson Laboratories (Bar Harbor, ME), were susceptible to VARV infection; subsequent pathologic, electron microscopic, tissue culture and immunohistochemical analyses confirmed systemic infection as the cause of mortality in mice infected with VARV following an approximate 13-day incubation period. We further characterized the hu-CD34 and BLT mouse strains by performing a VARV dosage study and determining viral trafficking using a serial sacrifice design; the PBMC strain was not used due to late disease progression and early development of graft-versus-host disease. Features of hu-CD34 and BLT humanized-mouse models may make

one or both of them suitable for testing the ability of potential antiviral therapeutics to protect mice following a VARV challenge. The BLT model study showed that infection is systemic by day 9 post infection, which is approximately 3–4 days before death of the animal; this time frame would allow for rigorous therapeutic testing in this animal model.

We recently completed a study using tecovirimat with the VARV humanized hu-CD34 model. Oral tecovirimat was given for 14 days starting either 0, 1, or 2 days post-VARV challenge. Preliminary results showed statistically significant but late protection for all tecovirimat treated groups compared to untreated controls. Extending the hu-CD34 study from 14 to 21 days and/or repeating the 14 day study with hu-BLT mice is the subject of a research project amendment submitted for review in this round. Further characterizing these VARV humanized-mouse models (for both hu-CD34 and BLT mouse strains) would allow regulatory bodies and the global community to assess their usefulness for evaluation of new smallpox medical countermeasures.

This work could also identify early biomarker(s) of smallpox (similar to those seen with human Ebola samples and NHP Ebola challenge studies). Identifying persons who are infected but asymptomatic could support focused use of medical countermeasures for treatment and non-pharmaceutical interventions to limit disease spread. We began biomarker molecular assays, which continued in 2021, using both MAGPIX and NanoString® technologies for the hu-CD34 and BLT models. Preliminary results showed a large down regulation of B-cell markers in the spleen, as well as transient down regulation and then upregulation of T-cell markers in the liver. Further work to characterize study samples could be useful to identify early/pre-symptomatic biomarkers of smallpox infection.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third meeting of the WHO Advisory Committee on Variola Virus Research in 2021.

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

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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) measures the ability of immune sera to neutralize mature virus forms and is used as a primary end-point for the evaluation of vaccines. Our prior studies, using sera from vaccinia virus (VACV)-vaccinees vaccinated with modified vaccinia Ankara (MVA-BN),[42] ACAM2000, Dryvax or LC16 vaccines, have indicated neutralization end-point titres may differ when using different species of target viruses. Variations in orthopoxvirus antigens likely account for these differences. New “third” generation vaccines derived from attenuated vaccine

[42] Commercialized by Bavarian Nordic as Imvamune® (Canada), Imvanex® (the European Union) and Jynneos® (the United States of America).

strains, such as modified Ankara vaccinia virus and LC16m8, could not be tested directly for efficacy against smallpox as most were developed near the end or after smallpox eradication. We have found a statistically significant difference in neutralization titres of vaccinee serum when using different target viruses (e.g. VARV as a heterologous target versus vaccinia virus as a homologous target). Therefore, the ability of sera to neutralize the mature virus form of VARV will provide an informative surrogate measure of efficacy, which is useful for evaluation of attenuated vaccines that do not elicit a major cutaneous reaction, the traditional measure of smallpox vaccine success.

In 2018, results of the VARV PRNT optimization study for MVA-BN vaccine were included in the Analytical Plan and Redevelopment Report approved by the United States Food and Drug Administration (FDA). Final titres in the study (n=100 per vaccine, 50 pre- and 50 post-vaccination taken at "peak" time-point for each vaccine) were presented at the twenty-first meeting of the WHO Advisory Committee on Variola Virus Research in 2019. Comparison of average fold-rise in titre, percentage of each regimen (ACAM2000 vs MVA-BN) reaching 4x and 8x titre rise, as well as geometric mean titre showed no statistically significant difference between vaccine regimens. These data requested by the FDA support the non-inferiority clinical trial data. The FDA approved MVA-BN in September 2019 for prevention of smallpox and monkeypox. During 2020, raw data of VARV PRNT assay runs were organized and archived for future availability and transparency.

CDC has also supported a MVA-BN vaccine study in the Democratic Republic of the Congo (DRC), where monkeypox is endemic. All serum from approximately 1000 vaccinated participants (MVA-BN liquid formulation; with blood collection on days 0, 14, 28, and 42, and at 6, 12 , 18 and 24 months) were tested for IgG and IgM by VACV enzyme-linked immunosorbent assay. Data cleaning and analysis is ongoing. Neutralization testing of a subset of participant serum against VACV and monkeypox virus (MPXV) show similar levels of seroconversion in all assays as in previous studies. Participants with previous/childhood vaccination had higher titres that were more durable through the two-year study, while the antibody response of naïve individuals peaked quickly after the second dose (Day 42) and then declined rapidly. In Part 2 of the study, which used a lyophilized formulation of MVA-BN, only the 12 month time-point was missed due to the coronavirus disease 2019 (COVID-19) pandemic. Specimens will be received and tested in 2021/2022. Studies to assess the longevity of VARV neutralization in a subset of sera will be based on VACV and MPXV neutralization results.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third meeting of the WHO Advisory Committee on Variola Virus Research in November 2021.

Use of live variola virus to maintain and regenerate non-infectious materials for diagnostic development support and to evaluate DNA diagnostics

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Variola virus (VARV) DNA and antigen stocks are useful for diagnostic development and validation. Understanding variability between VARV genomes is instrumental to understanding the sensitivity and specificity of nucleic-based diagnostic assays, and assay validation using extracted genomic DNA rather than plasmids which express only target portions of genomic DNA may be more robust. For sensitivity analyses, use of DNA from purified virions allows a calculation of the limit of detection.

Discovery of novel orthopoxviruses can also confound current diagnostic assays. For example, in 2015, an orthopoxvirus was discovered in Alaska (Alaskapox virus) that was not detected with the Laboratory Response Network (LRN) non-variola orthopoxvirus real-time polymerase chain reaction (PCR) assay. Cases of Alaskapox also occurred in 2020 (one case) and 2021 (two cases).

In 2021, diagnostic development focused on validation of new reagents and/or equipment as technology advances, to retain United States Food and Drug Administration (FDA) approval. Coronavirus disease 2019 (COVID-19) 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 techniques (focusing on platforms in use within state and local health laboratories) with approved orthopoxvirus assays. In 2021 we completed safety testing, allowing LRN laboratories to use two different large-scale extraction platforms for testing of environmental samples. These two buffers were able to inactivate monkeypox virus in tissue culture medium or tissue samples. Both buffers inactivated the virus, with failure of inactivation only when the virus was in 10-fold excess of the buffer. These data suggest that the extraction platforms could be used for clinical specimens in the future. Large scale extraction platforms would be critical for timely sample testing in a smallpox outbreak.

We also made progress with the portable Oxford Nanopore MinION sequencer. In 2019 we developed a wet laboratory protocol for sequencing 1-3 kb PCR amplicons and a portable bioinformatics pipeline for generating consensus sequences. We had several successful sequencing runs on a new device with disposable flow cells (called a Flongle), which improves biosafety and biosecurity when sequencing poxviruses. In 2021, monkeypox virus and other orthopoxviruses were sequenced directly from clinical samples; orthopoxvirus species and the monkeypox virus clade (West African or Congo Basin) were determined within 40 minutes of sequencing. Over the past year we have also continued to optimize our multiplex, real-time PCR assay for use on the portable GeneXpert System in collaboration with BioGX. In late 2020, we optimized the conditions for GeneXpert multiplex assay to differentiate VARV from other orthopoxviruses, and confirmed a limit of detection of ~3000 plaque-forming units using a mock clinical sample (VARV spiked on a swab).

In the USA, VARV is subject to the Select Agent Regulations (42 C.F.R. part 73).

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third meeting of the WHO Advisory Committee on Variola Virus Research in November 2021.

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

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Protein-based diagnostics will be critical for rapid detection and response to any re-emergence or reintroduction of 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. Conversely, inadequate or inaccurate point of care (POC) assays for antibody or antigen detection hastily adapted during epidemics can also lead to distrust and hamper public health efforts. These examples highlight the challenges of developing and deploying protein-based diagnostic assays. This report provides results from use of variola virus (VARV) and other orthopoxviruses (OPXV) to validate several protein-based diagnostic assays for accuracy and sensitivity. In the United States of America, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73).

CDC has completed assessment of monoclonal antibodies (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 remained the most sensitive for detection of OPXVs and that incorporation of a mAb with pAb led to trade-offs between sensitivity and specificity. Highly specific VARV mAbs in combination with pAb have shown detection of VARV around 5 X 10^4 PFU/mL, but low-level cross-reactivity with other OPXVs was observed with these combinations at concentrations higher than 1 X 10^7 PFU/mL. Although high-specificity mAbs have been developed, final selection and optimization of a POC assay format is incomplete. As cross-reactivity has been observed in many formats, we are pursuing multiplexed detection to identify the highest sensitivity and specificity achievable.

Previously we began collaborations with researchers at Arizona State University and with Tetracore® Inc. Arizona State University has developed a low-cost multiplex fluorescent lateral flow assay (LFA), which improved sensitivity by 2 – 3 orders of magnitude compared to traditional LFA formats. We are evaluating this fluorescent LFA using mAbs directed against vaccinia virus or monkeypox virus. 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 10^3 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 during the COVID-19 pandemic.

We have continued evaluating an OPXV-generic LFA for field use in the Democratic Republic of the Congo (DRC), where monkeypox is endemic, to detect monkeypox virus in patient skin lesion samples. The pilot study enrolled 36 participants in July 2021 (of 60 planned) with 34 (94%) of these patients having their results. Preliminary results found high specificity but sensitivity was low at 33% in specimens also tested by real-time PCR. There was a reduction in time from rash onset to test result from an average of over 30 days to 4.5 days, delays primarily related to the time required for specimen transport to the testing laboratory.

The progress detailed above under this proposal and plans for 2022 were reported at the twenty-third meeting of the WHO Advisory Committee on Variola Virus Research in 2021.

Abstracts from invited speakers

Testing a new class of anti-viral compounds: Restriction of orthopoxviruses by TRIM5

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One alternative to new drug development is to repurpose other approved drugs. The protein TRIM5α is a host restriction factor, best known for its role in restricting the replication of retroviruses, such as HIV-1, but not hitherto known to have activity against DNA viruses. It has been shown that vaccinia virus (VACV) infection causes downregulation of TRIM5α expression in the host cell.

A quantitative proteomic analysis of VACV-infected cells revealed the down-regulation of many host proteins involved in antiviral defense. Amongst these is the E3 ubiquitin ligase, tripartite motif protein 5 (TRIM5).[43] Using immunoblotting we confirmed VACV induces degradation of TRIM5 and showed this requires a single VACV protein C6, a multi-functional virulence factor.[44] In human cell lines lacking TRIM5, VACV plaque size and virus titre increased, confirming a role for TRIM5 in restricting VACV infection. Conversely, over-expression of TRIM5 reduced viral spread, showing TRIM5 is a poxvirus restriction factor. Prior reports showed that cyclophilin, a pro-viral host protein, is incorporated into VACV virions.[45] Furthermore, the approved drug cyclosporin A, an immunosuppressor and antagonist of cyclophilin A, inhibits VACV replication.[46] We confirmed these observations, and showed that inhibition of VACV replication by cyclosporin A is TRIM5-dependent.

Collectively, these results show: i) TRIM5 restricts VACV replication, and ii) VACV counters this restriction by C6-mediated degradation of TRIM5 and by incorporation of cyclophilin A into virions. Given that the C6 protein of VACV is highly conserved in both monkeypox virus (MPXV) and variola virus (VARV), these viruses may also degrade TRIM5 during infection. Similarly, because the structural proteins of VARV and MPXV are also conserved, it is likely that these viruses also package cyclophilin A into the virion to protect against TRIM5-mediated restriction. If so, these viruses may also be sensitive to cyclosporin A, or other known non-immunosuppressive inhibitors of CypA.

A project in collaboration with the United States Centers for Disease Control and Prevention, Atlanta, Georgia, USA is proposed to test if replication of MPXV and VARV is altered in cells in which TRIM5 or cyclophilin A are knocked out separately or together.

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43 Soday L et al., *Quantitative Temporal proteomic analysis of vaccinia virus infection reveals regulation of histone deacetylases by an interferon antagonist.* Cell Rep, 2019. 27: 1920-33 e7.

44 Unterholzner L et al., *Vaccinia virus protein C6 is a virulence factor that binds TBK-1 adaptor proteins and inhibits activation of IRF3 and IRF7.* PLoS Pathog, 2011. 7: e1002247; Stuart JH et al., *Vaccinia virus protein C6 inhibits type I IFN signalling in the nucleus and binds to the transactivation domain of STAT2.* PLoS Pathog, 2016. 12: e1005955; and Lu Y et al., *Histone deacetylase 4 promotes type I interferon signaling, restricts DNA viruses, and is degraded via vaccinia virus protein C6.* Proc Natl Acad Sci U S A, 2019. 116: 11997-12006.

45 Castro AP et al., *Redistribution of cyclophilin A to viral factories during vaccinia virus infection and its incorporation into mature particles.* J Virol, 2003. 77: 9052-68.

46 Damaso, C.R. and S.J. Keller, *Cyclosporin A inhibits vaccinia virus replication in vitro.* Arch Virol, 1994. 134: 303-19; and Damaso, C.R. and N. Moussatche, *Inhibition of vaccinia virus replication by cyclosporin A analogues correlates with their affinity for cellular cyclophilins.* J Gen Virol, 1998. 79: 339-46.

Further, the project will test whether these viruses show sensitivity to cyclosporin A, or related compounds, and if so whether this is TRIM5- or cyclophilin A-dependent. If results with MPXV are promising, it is proposed to then include VARV and further test whether compounds similar to cyclosporin A might have value as anti-orthopoxvirus therapeutics. The studies with monkeypox virus and variola virus, if successful, may identify a new class of potential anti-orthopoxvirus drugs.

These findings and plans for 2022 were presented at the twenty-third meeting of the WHO Advisory Committee on Variola Virus Research in November 2021.

Licensing and marketing update for brincidofovir

Odin Naderer
Chimerix®, Inc.

Oral tablet and suspension formulations of CMX001/brincidofovir (TEMBEXA®) were approved by the United States Food and Drug Administration (FDA) on 4 June 2021 for the treatment of human smallpox disease in adult and pediatric patients, including neonates. Brincidofovir is an oral antiviral formulated as 100 mg tablets and 10 mg/mL oral suspension dosed once weekly for two weeks. Brincidofovir impairs viral replication with a different mechanism of action than tecovirimat, which would offer an additional barrier to a smallpox emergence or bioterror attack. Work was ongoing to extend the recorded shelf-life of product formulations.

The approval of brincidofovir was based on the FDA animal rule which allows for testing of investigational drugs in animal models for diseases for which study in humans is not ethical or feasible (such as smallpox). Efficacy of brincidovir for orthopoxvirus disease was studied in two animal models of human smallpox disease, the rabbitpox model and the mousepox (ectromelia) model. In pivotal studies in each model, brincidofovir treatment resulted in a survival benefit compared to placebo. Chimerix has published a safety notice for this compound.[47]

As anticipated, the FDA issued a post-marketing "Field Study" requirement to evaluate tecovirimat in a smallpox outbreak. The design and need to collaborate with health agencies was outlined as follows:

Collaborate with US public health agencies to conduct a field study to evaluate the clinical response, drug concentrations, and safety profile of brincidofovir when used for the treatment of human smallpox disease due to variola virus infection. This trial should evaluate brincidofovir vs. tecovirimat vs. brincidofovir and tecovirimat combination therapy.

The study concept protocol has been developed and is being reviewed by a number of government agencies prior to further discussions with the FDA. Once the protocol is approved by the FDA, it will be deployed in the event of a smallpox outbreak in the United States of America.

[47] Further information is available here: Chimerix Receives U.S. Food and Drug Administration Approval for TEMBEXA® (brincidofovir) for the Treatment of Smallpox | Chimerix, Inc. (accessed 2 March 2022).

An additional post-marketing commitment was requested by the FDA to conduct cell culture studies to characterize brincidofovir antiviral activity against recombinant vaccinia viruses encoding specific amino acid substitutions that emerged in ectromelia virus in brincidofovir-treated mice.

We remain in discussions with the United States Biomedical Advanced Research and Development Authority (BARDA) regarding the possibility of supplying brincidofovir to the United States Strategic National Stockpile. This would meet the recommendations of Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) to have two smallpox treatments with different mechanisms of action in the stockpile. PHEMCE coordinates United States Federal efforts to enhance preparedness for chemical, biological, radiological and nuclear threats (CBRN) and emerging infectious diseases from a medical countermeasures perspective.

These updates and plans were presented at the twenty-third meeting of the WHO Advisory Committee on Variola Virus Research in November 2021.

Development of a pox monoclonal antibody cocktail – research, licensing & production update

Darryl Sampey
BioFactura, Inc.

BioFactura is developing a combination therapeutic designed to mitigate the risk of development of resistance and complement the existing small molecule antivirals approved by the United States Food and Drug Administration (FDA).

Consisting of a cocktail of monoclonal antibodies (mAbs), the Smallpox Biodefense Therapeutic (SBT) is intended to be specific for the treatment of smallpox disease. Using a stepwise approach BioFactura will pursue a development program to establish a controlled manufacturing process, a pharmacology, safety and efficacy profile in nonclinical animal models, and safety in a Phase I clinical trial in healthy human volunteers. This product development builds upon years of early research and development and a strong foundation of cell line, process analytical and manufacturing development experience.

Ultimately, BioFactura intends to seek FDA approval for the SBT as a treatment for smallpox disease. This work is supported by the United States Biomedical Advanced Research and Development Authority (BARDA).

These updates and plans were presented at the twenty-third meeting of the WHO ACVVRh in November 2021.

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