WHO ADVISORY COMMITTEE ON VARIOLA VIRUS RESEARCH

REPORT OF THE TWENTY-SECOND MEETING, GENEVA, 4–5 NOVEMBER 2020
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EXECUTIVE SUMMARY

The World Health Organization (WHO) Advisory Committee on Variola Virus Research (ACVVR) held its twenty-second meeting on 4–5 November 2020 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 authorized repositories at the Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Federal Service for Surveillance on Consumer Rights Protection and Human Well-being (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk Oblast, Russian Federation, 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. Twenty-six additional variola virus isolates held in the CDC collection had been sequenced. COVID-19 had delayed research during the year under review. The Committee recommended approval of four new proposals presented, two each from CDC and VECTOR, and continuation of previously approved projects.

Antiviral agents
Studies with tecovirimat were ongoing to meet regulatory authority post-marketing requirements. A paediatric formulation and an intravenous formulation were also under development. It was planned to assess tecovirimat in conjunction with vaccination with the approved modified vaccinia Ankara vaccine (MVA) in human volunteers. The safety of brincidofovir had been evaluated in clinical trials, with mild and reversible side effects, and regulatory approval was expected shortly. Study of analogues of ST-357, a possible second-line drug, had begun. VECTOR anticipated that phase 2 and 3 trials of NIOCH-14 would begin in 2021. VECTOR also proposed to test 15 new chemical compounds against variola virus in vitro. Both VECTOR and CDC continued to explore individual, or mixtures of, monoclonal antibodies. CDC’s humanized mouse model had displayed sensitivity to variola infection with a disease profile reminiscent of human infection.

Vaccines
Studies of the approved MVA vaccine 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 and a strong memory response in health workers two years after initial vaccination. Canada had extended indications for the vaccine to include prevention of monkeypox and other orthopoxviruses for persons at risk, making the MVA vaccine the first vaccine approved for a wider range of orthopoxviruses. The neutralizing antibody response of LC16m8, a third-generation vaccine licensed in Japan for prevention of smallpox, continued to be assessed. VECTOR continued development of VACΔ6, a 4th generation attenuated vaccinia vaccine, and phase 2 and 3 clinical trials were authorized; 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 suitable for a field setting. CDC was continuing 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 that both laboratories work towards developing test technology that would not require the use of live virus to validate, and that the advances observed during the COVID-19 pandemic could serve as a basis from which to further develop diagnostic techniques for orthopoxviruses.

Paleogenomics
Continuing previous discussions on the issue of research on human remains, where variola virus DNA may be the subject of investigation or an incidental finding, the Committee reviewed options for guidance, and recommended finalizing a risk assessment framework for handling of such DNA. WHO recommendations on the distribution, handling, and synthesis of variola virus DNA would be updated accordingly.

Conclusion
The Committee expressed support for the continued work on potential antiviral agents against smallpox, voicing concern about the time that may be required to develop monoclonal antibodies, and recommended that work continue on the vaccines and diagnostics in development. Regarding diagnostics, including point-of-care tests, the COVID-19 pandemic had led to the rapid development of technologies that may be applicable for orthopoxviruses, and the Committee urged researchers to propose alternative methods that would not require the use of live variola virus. Proposals presented prior to the meeting were recommended for approval. Other recommendations offered by the Committee are summarized in this report.
The twenty-second meeting of the World Health Organization (WHO) Advisory Committee on Variola Virus Research (hereinafter referred to as the Committee) was held at WHO headquarters, Geneva, Switzerland, 4–5 November 2020. The meeting was co-chaired by Dr Robert Drillien and Dr David Ulaeto. The agenda is included as Annex 1 and the list of participants as Annex 2. The meeting took place via video conference.

The objectives of the meeting were to:
- review progress of approved research with live variola virus and new research proposals; and
- update the research programme and recommendations for 2021–2022.

Dr Sylvie Briand, Director, WHO Global Infectious Hazards Preparedness Department, welcomed all participants to the meeting and spoke of the COVID-19 pandemic. She questioned if the world had been well prepared and how we could do better in the future, regardless of whether a pandemic was the result of a natural, accidental or deliberate event. Recently WHO had established a unit to look at accidental or deliberate events, and to assess risk arising from new technologies. Mentioning that this last year had witnessed the 40-year anniversary celebrations following the eradication of smallpox, she said that the advice of the Committee was still very relevant and appreciated. Finally, she spoke of the loss of longstanding Committee member Dr J. Michael Lane, saying that WHO would continue to honour his legacy.

Professor David Heymann, Chair, WHO Strategic and Technical Advisory Group for Infectious Hazards, thanked Dr Drillien and Dr Ulaeto for chairing this meeting, and talked of Dr Lane, with whom he had worked for two years in the nineteen seventies in India. Dr Lane had advised and counselled him and had been a wonderful person to work with, and the insight he brought to the Committee from his work during the smallpox eradication campaign had been exceedingly valuable. The Committee had been very important in overseeing the creation of medical countermeasures that would be needed in the event of re-emergence of smallpox or its use as a biological weapon. He spoke of monkeypox and its changing epidemiology and that it may one day fill the ecological niche left by smallpox, and the important potential of countermeasures developed for smallpox for the control of emergent monkeypox. He thanked this Committee for their input to the work on antivirals, vaccines and emergency programmes, and again mentioned his gratitude for the opportunity to express thanks for Dr Lane, who had led a very inspirational life.

Dr Rosamund Lewis of the WHO Smallpox Secretariat updated the Committee on events of the past year, thanking all members for their contributions and reviews of research proposals. Continuing with a membership update, she thanked Dr Willem Luytjes, member of the Committee for 12 years, who had now retired from membership. The Committee would receive an update on inspections of the variola virus repositories and review
progress in variola virus research for diagnostics, antivirals and vaccines as undertaken by the two WHO variola virus repositories. Dr Kazunobu Kojima, Technical Officer, WHO Health Emergencies Programme, said that two repository inspection reports had been published1,2, following which the Secretariat had reviewed detailed plans of action presented by VECTOR in the Russian Federation and CDC in the United States. Inspections were presently scheduled for 2021.

Continuing, Dr Lewis said that following discussions at the twenty-first Committee meeting in 2019, an informal working group had been set up to develop guidance on research on human remains where variola virus DNA might be found in order to review the WHO recommendations on the distribution, handling and synthesis of variola virus DNA3. Several articles had been published in this domain in recent years. In 2019/2020, biosafety/biosecurity risk assessments had been completed by researchers in collaboration with WHO for three projects, one with the University of Zurich4 and two with McMaster University.

Events of interest during the year included an occupational injury of self-inoculation with vaccinia virus in Pune, India, for which tecovirimat had been procured internationally from the manufacturer. Researchers in India had subsequently enquired about purchasing smallpox vaccine and therapeutics, but these were not available for purchase in small quantities as currently marketed only as products for stockpiling. In the Democratic Republic of the Congo, monkeypox was becoming more of a public health concern5; in 2020 there had been 6231 suspected cases, with both reported incidence and the case fatality ratio (CFR) having risen by about 50% compared to 2019. In August 2020, a second case of a novel human orthopoxvirus infection had been identified in Alaska, USA6.

Preparedness planning and continued engagement with partners on the health security agenda was ongoing, including stockpile planning for vaccines and antivirals and an internal training exercise for vaccine deployment. Strategies to improve access to medical countermeasures for monkeypox and other orthopoxviruses would be explored.

A year of events marking 40 years of smallpox eradication had been celebrated. For the occasion a stamp had been produced for Switzerland by the United Nations Postal Administration.

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Research on human remains

An update was presented by Dr David Ulaeto. Following the Committee meeting in 2019, a review of considerations regarding research on human remains where variola virus DNA may be found, also known as ‘ancient DNA’, had been undertaken by an informal working group. Guidance was needed because variola virus DNA had been found in a range of human remains. While several instances had been described, none had contained viable or intact virus. Genome sequencing of ancient variola virus remnants may be of public health benefit by adding to understanding of the evolution of viruses, including emergent zoonotic orthopoxviruses.

Current WHO recommendations advise that only the WHO repositories may hold more than 20% of a variola virus genome; exceptions are for fragments of less than 80 base pairs in length covalently bound to a matrix for use in developing diagnostics. The working group had discussed viability of the variola virus, and the limited risk related to discovery of variola virus DNA in ancient human remains as studies conducted to date had failed to uncover live virus.

Of interest were genomic studies on human remains, with or without suspicion of the person or persons having had smallpox, and studies of museum specimens suspected or known to have been from persons with smallpox. Dr Ulaeto presented options from the working group for the Committee to consider. Regarding human remains from known or suspected smallpox victims, the Committee considered options as to whether i) a proposed study would require permission from WHO, ii) such a requirement would be conditional on the date of interment, or iii) would instead require an internal institutional risk assessment with notification to WHO, and if risk was considered very low, then notification would be regarded as sufficient. Preference was given by the presenter to the third option. The Committee concurred and recommended that for research regarding human remains from persons known or suspected to have had smallpox, internal risk assessment be carried out by the research institution according to a WHO risk assessment framework.

Considering human remains with no prior suspicion of smallpox infection, no prior risk assessment for variola virus would be required. If variola DNA were to be identified, however, post hoc notification to WHO and an internal institutional risk assessment would be required using a WHO framework.

Similarly, considering smallpox museum specimens, where a range of different preservatives may have been used, the working group recommended and the Committee concurred that WHO should be notified and an internal risk assessment undertaken using a WHO framework. If the risk was considered to be very low, then the work could proceed. The Committee also considered the option that if, a priori, there were a possibility that over 20% of the variola virus genome would be found, authorization for the work should be reserved for existing WHO variola virus repositories, but found this option to be impractical and not necessary.

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With any sample of any provenance found to contain variola virus DNA, the Committee endorsed the proposal that if DNA fragments were one hundred or more base pairs in length on average, work should cease, and WHO advice be sought. Conversely, if the average fragment length was less than one hundred base pairs, work could continue and WHO should be informed. All materials should be inventoried and maintained in a biosecure manner. The Committee advised that if, after sequencing, DNA material was found to represent more than 20% of the variola virus genome, all remaining DNA-containing material should be destroyed (including all DNA fragment libraries). Original specimens from which samples were taken may be returned to the museum or for re-interment, according to the circumstances.

In discussion, the Committee further advised that 1), the WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA (2016) be updated to reference that a risk assessment was required for work with ancient DNA specimens suspected or subsequently discovered to contain variola DNA, and 2), that WHO develop a risk assessment framework for use by researchers, building on the format used in 2020.

Variola virus repository 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, and on the replenishment of stocks with non-infectious material derived from live variola virus required for diagnostics development

Dr Rinat Maksyutov reported that the variola virus collection at the VECTOR laboratories comprised 120 strains from Europe, Asia, Africa, South America and the Eastern Mediterranean with no change in the collection since the last report in 2019. The variola virus research laboratories at VECTOR were in compliance with national and international requirements and with WHO recommendations.

Research approved for 2020 and still planned for implementation included the: discovery and testing of novel chemical antivirals for smallpox treatment and prevention; assessment of the neutralizing activity of vaccinated volunteers’ sera and those of vaccinated animals against variola virus to support the development of less reactogenic 4th generation smallpox vaccines; and use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies.

Current proposals included two new requests: replenishment of stocks with non-infectious material, derived from live variola virus, required for diagnostics development; and development of advanced methods for rapid (point-of-care) diagnostics for orthopoxvirus infections.

Report on the variola virus collection at the WHO Collaborating Centre for Smallpox and other Poxviruses, Centers for Disease Control, USA

Dr Victoria Olson outlined the measures in place at the CDC smallpox BSL4 (biosafety level 4) laboratory, including the very restricted access and training procedures, and
provided an update on use of live variola virus. In February 2020, 20 original samples had been removed from long-term storage for DNA sequencing without propagation; the remainder of each sample was returned to long-term storage after processing. There were no complete withdrawals or additions to the long-term repositories.

In August 2020, a complete inventory of working stocks had been conducted. Since the last report in 2019, there had been: 36 uses (of 17 variola virus strains) to support nucleic-acid based diagnostics; 9 uses (of 3 strains) to determine the ability of tecovirimat and ST-357 to inhibit unique sequences of the virus target gene; 14 uses (of 2 strains) to support evaluations of monoclonal antibodies as potential antiviral agents; and 4 uses to characterize the humanized mouse animal model of variola virus infection.

Regarding phylogenetic analysis, 26 genomes had been newly sequenced this year in addition to the 142 sample sequences reported in 2019. The sequencing had been carried out without virus propagation; within the different clades there were differences in some gene sequences, e.g. F13L, but these did not seem to separate solely on geography. The annotation process had been initiated, after which it was expected that the sequences would be submitted to Genbank.

Earlier research with orthopoxviruses such as vaccinia had confirmed that a single amino acid change in the target protein VP37, encoded by the F13L gene, could lead to resistance of the virus to the antiviral agent tecovirimat. Of the 10 different variants of the variola virus F13L gene identified thus far, none had demonstrated this specific genetic change. It was proposed to complete analysis of the newly sequenced isolates to determine if additional F13L gene variants would be found and to use a cell line strategy for testing any isolates not available for tecovirimat screening (see below).

Members of the Committee discussed whether there was need to grow stocks of virus in the future, as with current technology it was now possible to obtain sequence information from the clinical samples that are in the collections. The researchers felt however that whole genome sequences were needed to test how diagnostics and antivirals would work. On questioning, it was learned that master seed stocks were kept at passage levels 2 or 3, and that little change had emerged in any of the viruses so far, although no full genome sequencing had been completed with serially passaged samples.

The Committee recommended that all further sequencing be done without culturing of virus (though there may be amplification of DNA) and that CDC should make all data available publicly as soon as possible.

**Research reports and proposals**

While progress had been made with some of the projects approved by WHO following the 2019 Committee recommendations, others were yet to begin due to delays related to the COVID-19 pandemic. There were four new requests to be considered from VECTOR and CDC.
Antivirals

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

Professor Sergei Shchelkunov reminded the Committee that preclinical studies with NIOCH-14 as a finished dose formulation (FDF) had shown the drug to be safe, bioavailable, and highly effective against orthopoxviruses when administered to animals orally.

In July 2020, the Ministry of Health of the Russian Federation had approved phase 1 trials to begin in September 2020; 90 persons (18–50 years old) had been recruited and the duration of the trial would be six months. Phase 2 and 3 clinical trials for NIOCH-14 were anticipated to follow in 2021–2022, and registration in 2023–2024.

In relation to the development of other antiviral compounds, work proposed for 2021 was testing of novel chemical antivirals for smallpox treatment and prevention. Fifteen compounds had been identified in 2019–2020 (containing monoterpenes or adamantane, as well as analogues of alkaloids containing fragments of amino acids), for which selectivity indices of 98 to 7340 had been established. These compounds would be tested in vitro for antiviral activity against variola virus.

The second proposed project involved the use of live variola virus to evaluate antiviral monoclonal antibodies. Four monoclonal antibodies to the variola virus proteins A30, B7, M1 and I3 had been generated in 2018–2020, and specificity to the target proteins had been demonstrated. Thus in 2021, it was expected to analyse the variola virus-neutralizing activity of these monoclonal antibodies.

In discussion, Committee members observed that good progress was being made on NIOCH-14 and other novel compounds.

CDC: Use of live variola virus to characterize effectiveness of the antiviral therapeutic tecovirimat (additional data), and of the novel antiviral therapeutic ST-357

Dr Christina Hutson reminded the Committee of the need for at least two antiviral agents in case of re-emergence of smallpox and outlined work on expression of the F13L amino acid variants in cell lines to investigate the antiviral activity of tecovirimat. As reported last year, 18 nucleotide haplotypes had been identified which translated to 10 amino acid variants; 7 of the 10 variants had been tested for sensitivity to tecovirimat using live variola virus.

For the three variants that were not available for testing, a cell line strategy would be employed. The cell line strategy included cloning of wild type F13L and use of polymerase chain reaction (PCR) mutagenesis to generate variants; this had been delayed due to the COVID-19 pandemic, but all approvals were now in place. Anticipated research with tecovirimat for 2021 therefore included creating stable cell lines expressing the variola virus F13L protein and evaluating sensitivity by infecting cells with a vaccinia virus recombinant lacking the F13L gene. Experiments would be conducted in collaboration with SIGA Technologies.
Regarding ST-357, the target and mechanism of action were distinct from tecovirimat or brincidofovir. It was a small molecular weight compound that targets the viral poly-A polymerase encoded by E1L and was active against a broad spectrum of orthopoxviruses (vaccinia, ectromelia, and cowpox in mouse models; variola virus in vitro) at nanomolar concentrations. Research however had identified solubility issues, and that the drug was rapidly metabolized in humans and non-human primates. The EC50s and EC90s in several strains of variola virus and vaccinia virus had been examined and although ST-357 had a promising antiviral target, it was necessary for medicinal chemists to design and synthesize more suitable preclinical candidates.

A SIGA/US National Institute of Allergy and Infectious Diseases (NIAID) medicinal chemistry campaign had produced 21 analogues by May 2020, with demonstrated increase in aqueous solubility, and improved metabolic profile across species. Research proposals for 2021 therefore were to propagate variola virus (if needed) to attain sufficient material for antiviral screening, and to determine the EC50s of ST-357 analogues as preclinical candidates. Committee members observed that ST-357 could be a promising drug candidate if problems with solubility could be overcome.

Tecovirimat: Commercial research, licensing and production update

Dr Dennis Hruby reported that post-marketing activities for tecovirimat included developing a paediatric formulation, as well as characterization of its activity against a panel of variola virus isolates and recombinant vaccinia viruses.

A marketing authorisation application (MAA) had been submitted in 2020 and was now under review, and in Canada a new drug submission (NDS) was in progress for tecovirimat. Requested indications for other orthopoxviruses included monkeypox, cowpox and vaccinia viruses. Indications were also to be revisited with the FDA. In the US, more than two million treatment courses had been stockpiled and re-supply would proceed as per the expiry date. In Canada, the drug had been supplied to the Department of National Defence and would be supplied to the Public Health Agency of Canada.

A clinical study of treatment of monkeypox with tecovirimat in the Central African Republic was being developed in collaboration with the University of Oxford. Compassionate use cases had so far included vaccinia (six cases in US, one case in India) and cowpox (four cases in Europe).

In preclinical studies in mice and non-human primates of pre-exposure combinations of tecovirimat and ACAM2000 smallpox vaccine, tecovirimat administration had not reduced vaccine efficacy. There remained a security gap in the days following exposure to smallpox. Earlier preclinical post-exposure prophylaxis studies combining use of tecovirimat with either ACAM2000\(^8\) smallpox vaccine or with MVA-BN\(^9\) in non-human primates had been completed. In November 2019, the FDA recommended a tecovirimat plus MVA–BN clinical immunogenicity study to verify that tecovirimat administration would not interfere with the immune response to the vaccine; a clinical study phase 2 protocol was under review at FDA.

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8 A licensed replicating live smallpox vaccine manufactured from vaccinia cell cultures by Emergent BioSolutions Co Ltd.

9 The modified vaccinia Ankara smallpox and monkeypox vaccine by Bavarian Nordic.
Regarding the paediatric formulation under development, a powder for reconstitution was being developed for patients weighing 13kg or less. Prototype products recently manufactured would be evaluated in a pharmacokinetic (PK) clinical study and the selected prototype would be further developed and compared with the capsule product in a clinical study. A New Drug Application submission was planned in the following years.

The intravenous formulation of tecovirimat being developed for smallpox in patients unable to benefit from the oral capsule was formulated as a single-use vial, in a dose of 200 mg twice daily via 6-hour IV infusion for up to 7 days. Toxicology and efficacy studies had been completed, and in clinical studies no serious adverse events or deaths had been reported. A new drug application and commercial manufacturing were both scheduled for 2021.

**Brincidofovir: Research, licensing and production update**

In assessing brincidofovir for treatment of smallpox, Dr Odin Naderer reported that Chimerix had undertaken a comprehensive programme: efficacy and PK studies under the animal rule, clinical safety and clinical pharmacology studies, and human dose justification. The Committee was reminded that Brincidofovir is the lipid conjugate of cidofovir (CDV) and was designed to improve the cellular uptake of CDV to enhance the antiviral concentration of the active moiety, cidofovir-diphosphate.

Two pivotal efficacy and PK studies had been carried out in the rabbitpox and mousepox (ectromelia) models; statistically significant improvement in survival was seen in these models when compared to placebo. Safety had been evaluated in over 1600 human subjects in clinical trials. Analysis of the short-term safety of brincidofovir had demonstrated that treatment with 200mg weekly for two weeks was generally well tolerated, with some gastrointestinal complaints and transient increases in hepatic transaminases. Chimerix was expecting to gain approval from FDA in the following year.

**Use of live variola virus to evaluate monoclonal antibodies as antiviral agents**

Dr Ashley Kondas noted that, as endorsed by the Committee in the previous year, it was felt that monoclonal antibodies (mAbs) might provide a suitable alternative to tecovirimat as they had a different mechanism of action from tecovirimat, and lower probability that a single amino acid change would provide resistance. CDC was working with four different developers to assess monoclonals.

Vanderbilt Vaccine Center had created a mixture of monoclonal antibodies (known as Mix4), but in vitro testing revealed the individual mAbs within Mix4 did not perform well against the extracellular enveloped virion (EEV) forms of variola and monkeypox. So in 2020, work was instead focussed on developing a universal mix which could neutralize variola, monkeypox, vaccinia and cowpox viruses, but the COVID-19 pandemic had caused significant delays. In 2021, efforts would be focussed on characterizing neutralization ability for ten of the original 48 mAbs developed.

Data from the second developer had been presented previously. With a cocktail of two mAbs (one intracellular mature virion [IMV] and one EEV), neutralization was seen with
both the individual mAbs and the mix. The next steps would be humanization of the mAbs and retesting in neutralization assays.

A third developer had established a cocktail of two mAbs which were efficacious in two non-v variola animal models. However, results were less than optimal when tested in a non-human primate monkeypox study by Public Health England.

Results from a fourth developer with a cocktail of three mAbs were presented previously. Investigation had identified limitations of one mAb to neutralize variola virus despite its strong monkeypox virus neutralization. In late 2019, the developer was awarded a development contract for its Smallpox Biodefense Therapeutic, and humanization of the mAbs was completed in September 2020. However, evaluation of protection provided by the mAb cocktail within different non-v variola animal models had given conflicting results.

CDC was proposing to include in its proposals for 2021 other neutralization assays (e.g. comet reduction assay) to evaluate mAbs, in addition to the current plaque reduction neutralization test (PRNT), and to continue the testing of mAbs and mixes.

In discussion, the Committee noted that much work remained to be completed. A mAb mix would be a hospital solution only, as an adjunct for very sick patients. Monoclonal antibodies were very safe, but up to ten years may be needed to complete development.

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

Dr Kondas presented the case for identifying a suitable mouse model for human smallpox. The work had been partially approved in 2019/2020 to complete a previously approved study, and an updated proposal for 2020/2021 had been submitted to the Committee. As no animal model replicated the course of human infection with variola virus, it was thought that the humanized mouse might provide such a model. In 2015, three types of humanized mice had been studied. Mortality was high; all three strains of mice were very highly susceptible to variola virus. Hu-BL T and hu-CD34 mice were selected for further characterization. There was no significant difference between the two regarding mortality or viral load, but hu-BL T mice were more likely to seroconvert while hu-CD34 mice had the longer lifespan.

In these mice, variola virus was first detected in the nasal cavity; by day 4 virus DNA was detectable in the blood, and by day 9 there was complete systemic infection. This information could serve to assess whether a drug was capable of halting the spread of variola virus infection and evaluate medical countermeasures at different stages of disease. There was need to validate the model, and as tecovirimat was already licensed it would be logical to use this drug to confirm utility of the model. Mortality data would be compared with other surrogate animal models. If tecovirimat was successful, it would provide confidence for using the mouse model with other drugs such as monoclonal antibodies.

In discussion, it was learned that both mouse models were to be tested one at a time. On questioning about how well the model correlated with the human disease, it was reported that some mice in both groups had developed a secondary bacterial infection
despite being maintained under sterile conditions. The same inoculum had been used, and there were negative controls in every study, but these latter did not develop the secondary infection. The only mice to develop secondary bacterial infection were those that had first developed a robust variola virus infection, which was similarly seen during human viral infections. While the model was not perfect, there was good correlation with human smallpox. Some Committee members questioned whether the modified mouse model was needed, as one drug (tecovirimat) was approved and brincidofovir and NIOCH-14 were moving towards approval. Others expressed satisfaction that the model seemed to be proving useful.

Discussion on antiviral research projects

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

The Committee considered that the proposed phase 2 trials of tecovirimat with the MVA-BN smallpox vaccine, to explore post-exposure prophylaxis options and assess whether the treatment would affect development of an immune response, was a novel idea to be encouraged.

In deliberating the humanized mouse model, the majority of members recommended approving the project. Now that the work had proceeded so far, the model should be tested with tecovirimat to establish its utility in, and a baseline for, evaluating novel medical countermeasures to halt variola virus infection. If the model worked, any new drug could be assessed with it; there were however reservations about using the model to test monoclonal antibodies.

In considering how far the work on brincidofovir had advanced, and that approval was expected shortly, the agreed goal of two antiviral drugs for smallpox may be reached in the coming year, so this outcome would need to be addressed at the next meeting of the Committee and presented to the World Health Assembly.

The Committee noted that research on mAbs had not yet produced tangible results and that 5 to 10 years was needed before a product would be ready. In this regard, the Committee felt that CDC could use its resources more effectively, and that, given some of the work may be duplicative and the research needed to be accomplished in as short a time as possible, CDC could explore collaborating through a consortium model with the companies with which it is involved to improve efficiency in research and development.

The Committee observed that research and licensing efforts had moved forward in relation to other orthopoxviruses; this was a welcome advance in exploring the wider public health benefits of smallpox medical countermeasures. Members introduced the issue of cost of, and access to, the products arising from research with live variola virus; and recommended that researchers be reminded that their research, as authorized by the World Health Assembly, is for the benefit of humanity, and to consider cost and access as essential components of their work.
Thus the Committee recommended:

- to continue studies on NIOCH-14, fifteen new chemical compounds and four monoclonal antibodies at VECTOR.
- to continue studies on ST-357 and complete the study of tecovirimat activity against different F13L isolates at CDC.
- that further studies with tecovirimat should not involve use of live variola virus, except for testing the humanized mouse model, as the cell line strategy can be used for testing additional F13L sequences and all other necessary studies had been completed.
- to continue assessment of the humanized mouse model with tecovirimat.
- to continue studies on monoclonal antibodies and mixes at CDC as per the revised protocol provided to WHO pursuant to discussions during the meeting.
- that CDC be encouraged to enter into a consortium with the entities with which it is involved in monoclonal antibody development to improve efficiency and collaboration.

In conclusion, the four proposals received for the Committee’s advice were recommended for approval by WHO by a majority of the members who provided individual reviews in advance and by consensus following discussions at the meeting. The projects previously approved by WHO and presented as still in progress or yet to begin were endorsed for continuation.

The Committee further recommended that tecovirimat be included in the WHO emergency stockpile, along with preparation of WHO Emergency Use Listing as needed if also intended for orthopoxviruses other than smallpox, and efforts made to make the product available to the wider global community.

**Vaccines**

**VECTOR: Clinical trials of the anti-smallpox vaccine VACΔ6**

Dr Maksuytov reminded the Committee that preclinical studies in 2019 on the fourth-generation non-adjuvanted smallpox vaccine VACΔ6\(^{10}\) had shown that the protective properties of the vaccine were not inferior to the reference vaccine used\(^{11}\). In 2019, phase 1 clinical trials had commenced in volunteers aged 18 to 40 years. Evidence of local and systemic reactions, including in blood, urine, allergic reactions, immunological response, and virus shedding were compared with prior experience following use of the reference vaccine. VACΔ6 was associated with the generation of antibodies to orthopoxviruses in the sera of volunteers.

In July 2020, the Russian Federation authorities approved the conduct of phase 2 and 3 clinical studies with VACΔ6. The study design was a randomized, open, comparative study in parallel groups to study the protective efficacy of VACΔ6. The second stage was to conduct a double-blind, comparative, randomized, placebo-controlled study in parallel groups. Three hundred and thirty-four volunteers aged 18 to 60 years had been enrolled.

\(^{10}\) Based on a highly attenuated strain of vaccinia virus with the alteration of six virulence genes.

\(^{11}\) A live smallpox vaccine commercially available in the Russian Federation.
It was proposed to assess, in 2021, the levels of variola virus neutralizing antibodies in volunteers vaccinated with VACΔ6 and those immunized with a conventional cutaneous vaccine, and to compare the results with vaccinia virus neutralization. Similarly, levels of variola virus-neutralizing antibodies in the sera of vaccinated animals would be evaluated.

If successful, marketing authorization would be achieved in 2021–2022. As well, it was proposed to obtain additional data on the levels of variola virus-neutralizing antibodies in immunized volunteers and animals with several new modified vaccinia virus variants. In the future, VACΔ6 might be licensed for other orthopoxviruses in humans, such as monkeypox.

The Committee agreed to recommend continuation of the project; it had been approved by WHO following the previous meeting but delayed due to COVID-19.

**CDC: Study of MVA-BN vaccine in health workers in Tshuapa Province, Democratic Republic of the Congo**

Dr Hutson reported on the vaccination of health workers against monkeypox with MVA-BN, in an ongoing study in Tshuapa province, in the Democratic Republic of the Congo. Monkeypox incidence had been rising in West and Central Africa: in 2019, more than 5000 suspected monkeypox cases had been reported from 18 different provinces, with at least 100 deaths. Enhanced surveillance in Tshuapa province over several years had yielded samples from about 500 suspected cases per year of which about 300 had been confirmed each year to be monkeypox. The cohort study in Tshuapa province was being carried out to evaluate the safety and immunogenicity of MVA-BN in a field setting; it was a collaboration between the ministry of health of the Democratic Republic of the Congo, the Kinshasa School of Public Health, The International Communication and Education Foundation (INCEF), and CDC.

The vaccine had been administered to study participants on days 0 and 28, with visits to each participant at various timepoints up to 2 years post-vaccination, when blood was sampled. Diaries for adverse events and exposure were completed throughout the study to document contact with monkeypox disease patients and disease occurrence.

In the first cohort, a liquid frozen formulation of the vaccine had been administered to the 1000 or so participants. No serious adverse events were seen; however, one of the enrolled health workers had developed monkeypox about 2.5 years following vaccination. In the second cohort, a lyophilized formulation of the vaccine had been administered to the 600 enrolled health workers; this study was ongoing and, as of 4 November 2020, no case of monkeypox had been identified, nor any severe adverse events.

Regarding assessment of the immune response in the first cohort, nearly 7000 samples were available for testing. Enzyme linked immunosorbent assay (ELISA) tests had been completed, with preliminary IgG and IgM analysis. The seroconversion rate for IgM was 24%, and for IgG was 99%. For IgG, peak titre was seen on day 42 for both naïve and

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prior-vaccinated groups. Overall, the seroconversion rate was comparable to previously reported results. Assessment of the neutralizing antibody response was under way. Study of the second cohort was ongoing to assess immunogenicity, neutralizing antibody response, duration of immunity and safety.

For 2021, the plan was to initiate testing on the sera of MVA-BN vaccinees obtained six months or more post vaccination, and to also assess the sera against live variola virus. The samples had been collected and screening against vaccinia virus using the ELISA test had begun. Sample selection for screening against variola virus would be done after vaccinia virus/monkeypox virus neutralization assays are completed. Evaluation of the capacity of sera to neutralize the IMV and/or EEV forms of different strains of live variola virus, sampled from subjects' long term after MVA-BN vaccination, would help inform policy for use of the vaccine.

The Committee agreed to recommend continuation of the project; it had been approved by WHO following the previous meeting but delayed due to COVID-19.

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

**Dr Heinz Weidenthaler** spoke of the growing number of human monkeypox infections seen in endemic countries; WHO data from the Democratic Republic of the Congo had indicated that the number of human cases and case fatality appeared to be increasing\(^\text{13}\) (4594 suspected cases to September 2020, with a case fatality ratio [CFR] of 3.7%, compared to 3794 suspected cases and a CFR of 1.9% during the same period in 2019).

Results of a phase 3 non-inferiority clinical trial comparing the MVA-BN vaccine with ACAM2000 had been published in 2019\(^\text{14}\). MVA-BN was shown to be non-inferior; no safety concerns associated with the vaccine had been identified, and the immune responses suggested that MVA-BN would protect against variola infection.

Investigations were ongoing with use of the vaccine to prevent human monkeypox in health workers in the Democratic Republic of the Congo. So far, the MVA-BN vaccine had shown an excellent safety profile, with a strong memory response two years after initial vaccination. The vaccine was easy to administer (by subcutaneous injection). The vaccine currently licensed in several jurisdictions is a liquid-frozen formulation; the freeze-dried formulation was expected to complete clinical development early next year.

The Committee felt the vaccine could be a powerful tool for dealing with a smallpox outbreak. For monkeypox, prevention where feasible would be a step forward and strategies could include a vaccine such as this. Committee members recommended making the vaccine widely available and discussed options to promote its manufacture in other countries which have capacity (e.g. India, China, Botswana).

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**LC16m8 smallpox vaccine: Research, licensing and production update**

Dr Yasuhiko Shinmura, from KM Biologics Co. Ltd of Japan, presented an update of their freeze-dried attenuated smallpox vaccine, LC16m8, which had been licensed in Japan in 1975 for smallpox prevention. The shelf life of bulk vaccine substance was 5 years when stored at 80°C, and 4 years for the final product stored at 20°C. KM Biologics continued to manufacture the vaccine in accordance with the national strategic stockpile needs in Japan.

Comparison of the neutralizing antibody response between LC16m8 and Dryvax\textsuperscript{15} vaccinees had continued during the year; due to COVID-19, data analysis had not yet been completed. However, preliminary findings indicated the efficacy profile of LC16m8 to be equivalent to Dryvax. In collaboration with CDC, it was planned to evaluate the neutralizing antibody response against several strains of variola virus in both the LC16m8 and Dryvax vaccinees.

It was not planned to change the shelf life of 4 years, although a longer shelf life would facilitate stockpile management. The LC16m8 vaccine was available for the limited use of the Japan Self Defence Forces, health workers and other personnel deployed to orthopoxvirus endemic regions of the world, and scientists and laboratory personnel at risk of exposure to orthopoxviruses. The vaccine was not available to the general public as not recommended for the general population except for smallpox outbreak incidents. Safety of the vaccine had been confirmed in children, and in immunocompromised animal models, but vaccination had not yet been recommended for use in immunocompromised persons.

**Diagnostics**

**VECTOR: Development of advanced methods for rapid (point-of-care) immunochemical diagnostics of orthopoxviruses in clinical specimens**

As presented during the twenty-first Committee meeting, Professor Shchelkunov 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, and 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 unpurified samples within the concentration range of $10^3$ to $10^4$ pfu\textsuperscript{16}/ml. The assay was specific and easy to use and could be used in a field setting.

The design and manufacture of the kit's accessories would be optimized in 2021, and the kit tested using a wide range of orthopoxvirus species including variola virus and monkeypox virus. The proposed study was “Development of advanced methods for rapid (point-of-care) diagnostics of orthopoxvirus infections”.

The proposal to replenish stocks of non-infectious material, derived from live variola

\textsuperscript{15} A vaccine derived from the New York City Board of Health vaccinia strain, grown in calf lymph culture.

\textsuperscript{16} Plaque forming units.
virus, would meet the need to support ongoing diagnostics development. In general, the Committee was favourable to the proposals.

**CDC: Use of live variola virus to maintain and regenerate non-infectious variola virus derived materials for diagnostics development support, and to develop protein-based diagnostic and detection assays specific for variola virus**

**Dr Olson** reported that CDC was continuing its 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 Biomédicale (INRB) in Kinshasa, to improve both nucleic acid-based and protein-based rapid diagnostic tests. Both types of test had advantages and disadvantages, as outlined during the twenty-first meeting of the Committee.

Some progress in adapting nucleic acid-based diagnostic assays to a more “field deployable” setting had been made. The novel “snap-tip” delivery system for the cartridge-based nucleic acid amplification test (NAAT)\(^\text{17}\) had been optimized, and by adjusting the annealing temperature and buffer used in the cartridge, the assay efficiently detected variola virus in contrived clinical samples, though at higher concentrations than the previously established limit of detection for individual real-time PCR assays. Work would continue to improve detection. Two laboratories – in Boende, Tshuapa province and Kisangani, Tshopo province – had been assessed for capability to conduct monkeypox assays in remote settings. One advantage of the cartridge-based NAAT system was the minimal sample processing required prior to its use for diagnosis.

Protein-based tests provided valuable flexibility for field deployment. Variola specific mAbs had been generated and optimal combinations identified, with promising detection of variola virus in a lateral flow assay (LFA) format. Evaluation of a commercial LFA (Tetracore) for monkeypox detection in the Democratic Republic of Congo was ongoing; participants would continue to be enrolled during 2021 (18 enrolled to date, of an anticipated 60). Preliminary experience had shown reduced time for confirmation (3.5 days as opposed to 35 days), but sensitivity was low.

A question was raised about the safety of technicians in regard to protein-based assays, where there was no inactivation of sample (as opposed to nucleic acid-based assays). As the technicians conducting the LFA wore the same personal protective equipment (PPE) as used for sampling a patient for national surveillance, and there were very few manipulations to be done, and afterwards the PPE was discarded as contaminated waste, the technicians were felt to be in no specific danger. There was discussion about emerging technologies and the new portable devices that were appearing for nucleic acid-based assays in the context of COVID-19; perhaps now was the right time to endorse further exploration of diagnostics.

In general, the Committee was favourable to the proposals.

\(^{17}\) GeneXpert® System.
Discussion of diagnostics research updates and plans

Members of the Committee discussed various aspects of diagnostics; their observations and suggestions are presented below.

While the main drivers for the development of diagnostics had been concern over smallpox bioterrorism and more recently synthetic biology, it was now clear that monkeypox was taking on a higher profile than previously. Smallpox remained a concern, and research was clearly helping in developing countermeasures for monkeypox which could otherwise be considered a neglected disease. There were also other emerging orthopoxviruses to consider.

The Committee was in general agreement that the abovementioned studies should be completed but a main continuing concern was whether live variola virus needed to be used. Considering the COVID-19 pandemic, there had been no immediate need for virus sharing to begin diagnostics development, which began as soon as the genetic sequence of the SARS-CoV-2 virus had been made available. For nucleic acid-based systems, only sequences would be needed to make templates.

The COVID-19 pandemic had motivated significant developments in the field of diagnostics. Nucleic acid amplification tests, for example: some had been adapted by changing the primers included or by adapting already existing technologies. It was worth exploring in the coming years how orthopoxvirus diagnostics could benefit from these techniques. Some members strongly endorsed the exploration of nucleic acid-based detection methods that did not require sophisticated temperature control, such as the LAMP isothermal amplification technique \(^\text{18}\) and other examples from COVID-19 as provided by a member of the Committee\(^\text{19,20,21,22,23,24}\).

The other main concern was protein-based diagnostics. For smallpox, the researchers felt that protein-based diagnostics still needed to be tested on inactivated as well as on live virus to ensure validity. Could surrogates for live virus be found for protein-based diagnostics? The Committee recommended that researchers search for alternative techniques that did not involve the use of live variola virus.

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In the Democratic Republic of the Congo, the use of protein-based diagnostics had reduced the time to confirmation in a remote setting, which could nonetheless still require days. Could the sensitivity, specificity and simplicity of protein-based diagnostics be bettered to make them more field deployable? It was felt that lateral flow assays, for example, could be cost effective.

Some members also remarked that the ability to diagnose disease outbreaks at the point of occurrence should, by now, have been in place. While significant progress had been made by VECTOR and CDC on a number of candidate surveillance and point-of-care tests, there remained many hurdles to overcome before such tests would become widely available. WHO and partners were urged to make essential efforts to expedite availability and use of tests in the field.

The diagnostics field was moving rapidly and new methods would be found for COVID-19 which could be considered for application to smallpox. Members strongly advised the researchers to explore innovative methods that brought together sensitivity, specificity and usability as a point-of-care diagnostic and to chart a path to ceasing the use of live variola virus.
PROGRAMME OF RESEARCH

The purpose of the research overseen by the WHO Advisory Committee on Variola Virus Research, 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 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 to 2022 outlined at the twenty-first meeting of the WHO Advisory Committee on Variola Virus Research was presented in the report of the twenty-first meeting. In 2020, 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 timeframe. Minor adjustments are highlighted in the table below, 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-second meeting are summarized in the section that follows the table.
**Table. Programme of research using live variola virus presented by WHO variola virus repositories for 2020 to 2022 (with updates)**

<table>
<thead>
<tr>
<th>Area of work</th>
<th>United States Centers for Disease Control and Prevention (CDC)</th>
<th>State Research Center of Virology and Biotechnology (VECTOR), Rospotrebnadzor, Russian Federation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genome sequencing</strong></td>
<td>Complete genomic sequencing of 20 strains <em>(updated to 22 strains)</em> with adequate epidemiological information.</td>
<td>Complete the genome sequencing of the remaining 88 strains.</td>
</tr>
<tr>
<td><strong>Diagnostics</strong></td>
<td>Adapt and optimize multiplex nucleic acid tests for new platforms <em>and field settings</em>. Continue development and optimization of protein-based tests.</td>
<td>Optimize the design of the immunochemistry test kit and its accessories using orthopoxviruses, including variola and monkeypox viruses.</td>
</tr>
<tr>
<td><strong>Vaccines</strong></td>
<td>MVA-BN and LC16m8 Finalize <em>efficacy</em> testing on long-term titre samples from MVA-BN and/or LC16m8 vaccine trials (as samples are available).</td>
<td>VACΔ6 Complete Phase 1 clinical trials (adults 18–40 years) by Dec 2019. Undertake Phase 2 and 3 clinical trials in 2020–2021 and assess variola virus neutralizing antibody titres from sera of participants. Complete registration in 2022.</td>
</tr>
<tr>
<td><strong>Animal models</strong></td>
<td>Humanized mouse models Complete the remaining in vitro work on the HU-BLT model. Continue to assess Hu-BLT and Hu-CD34 models using tecovirimat <em>(proposed in 2019, not approved; resubmitted and approved in 2020).</em></td>
<td></td>
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</tbody>
</table>

*edits in italics are new from November 2020 compared to the plan set out in 2019.

NB: This multi-year research agenda was presented to the WHO Advisory Committee on Variola Virus Research at their twenty-first and twenty-second meetings in individual presentations. Proposals were reviewed by Committee members in order to provide recommendations to WHO as outlined further in this report.
RECOMMENDATIONS

In a closed session for Committee members and advisors, the pros and cons of the new proposals were discussed (see above in the respective sections and recommendations below). The Committee also endorsed continuation of the several projects that had been approved by WHO in 2019 and were ongoing but delayed by COVID-19. Last year a three-year roadmap had been outlined for review, and minor adjustments to this have been outlined above.

The new context of the COVID-19 pandemic adds an element of urgency to considerations of, firstly, the cost and accessibility of countermeasures developed for smallpox, and secondly, the importance of continuing to broaden the potential benefits for prevention and control of other orthopoxviruses.

Notwithstanding this new context, members of the Committee nonetheless advocated that the World Health Assembly position to limit the use of live variola virus to remaining essential research be earnestly observed. It was possible that the coming year would see approval of a second smallpox antiviral agent. There was however concern that development of monoclonal antibodies might take years to complete. The WHO variola virus repositories, CDC and VECTOR, should make plans to move forward approved research and develop alternatives to the use of live virus, most particularly and rapidly in the domain of diagnostics development, and to have a roadmap towards complete cessation of its use.

The following section summarizes the recommendations of the Advisory Committee on Variola Virus Research as articulated at its twenty-second meeting.

General recommendations

- Make sequence data for all available isolates publicly available as soon as possible.
- Complete all further variola virus DNA sequencing without amplification of virus.
- Continue research and development of antivirals, vaccines and diagnostics that had been recommended previously by the Committee in 2019 but delayed in 2020 by COVID-19.
- Remind researchers that their work is for the benefit of humanity and to consider cost and access.
- Ask collaborating centres to search for alternative techniques in the future that did not use live virus, including through employing surrogate viruses.
- Limit the use of live variola virus to any remaining essential research and to finally terminate its use.

Antivirals

- Continue the studies on NIOCH-14, fifteen new chemical compounds and four monoclonal antibodies (VECTOR).
- Continue studies on ST-357, as well as monoclonal antibodies and mixes (CDC).
- Refrain from using live virus in any further studies with tecovirimat (except as below, to test the humanized mouse model).
• Develop the humanized mouse model using tecovirimat to establish a baseline (not
to test tecovirimat, but to assess the mouse model).
• CDC to explore building a consortium with their partnering entities to improve
efficiency and collaboration in developing monoclonal antibodies.

Vaccines

• Conduct Phase II clinical study of MVA-BN to assess immunogenicity in the
  presence of treatment with tecovirimat.
• Encourage parties to make third and fourth generation vaccines widely available,
  reviewing indications and considering manufacturing options in countries with
  capacity.

Diagnostics

• Explore nucleic acid-based detection methods that do not require sophisticated
  temperature control.
• Invite researchers to develop methods that do not require the use of live variola
  virus.
• Expedite the availability of orthopoxvirus diagnostic tests in the field.

Paleogenomics

• Develop guidance and a risk assessment framework concerning research with
  human remains that might contain variola virus DNA.

Emergency preparedness and public health benefit

• Make tecovirimat available to the wider global community and include it in the
  WHO emergency stockpile.
Annex 1 | Agenda

**Twenty-second meeting of the Advisory Committee on Variola Virus Research**

**Final Agenda**

**Objectives of the meeting**
- Review progress of approved research with live variola virus and new research proposals
- Update research programme and recommendations for 2021–2022

**Wednesday, 04 November 2020 – Day one**
Chair: Dr Robert Drillien, Chair, ACVVR22

*Video link: https://who-e.zoom.us/j/98453673344*

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 1. Opening and report of the WHO Smallpox Secretariat</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:30</td>
<td>Opening remarks</td>
</tr>
<tr>
<td></td>
<td>Dr Sylvie Briand, Director, Infectious Hazards Preparedness, Health Emergencies Programme</td>
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<td></td>
<td>Dr David Heymann, Chair STAG-IH</td>
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<tr>
<td>13:45</td>
<td>Roll call, declarations of interest</td>
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<tr>
<td></td>
<td>Report of the Smallpox Secretariat</td>
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<td></td>
<td>Dr Rosamund Lewis, WHO Smallpox Secretariat</td>
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<tr>
<td>13:55</td>
<td>Update of WHO recommendations on handling, distribution and synthesis of variola virus DNA and guidance for researchers</td>
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<td></td>
<td>Dr David Ulaeto, Principal Scientist, DSTL, UK</td>
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<tr>
<td>14:10</td>
<td>Discussion</td>
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<td>All</td>
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</table>

**Session 2. WHO Collaborating Centre reports - Variola virus and DNA collections (breakout room)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 1. Opening and report of the WHO Smallpox Secretariat</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:20</td>
<td>Summary of proposals for research with live variola virus</td>
</tr>
<tr>
<td></td>
<td>Dr R. Drillien, Chair, ACVVR</td>
</tr>
<tr>
<td>14:30</td>
<td>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, Rospatrebmadzor and Replenishment of stocks with non-infectious material, derived from live variola virus, required for diagnostics development Approved 2018/2019; Proposed 2020/2021</td>
</tr>
<tr>
<td></td>
<td>Dr R. Maksyutov, Director General, FBRI SRC VB VECTOR, Rospatrebmadzor, Russian Federation</td>
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</table>
### Session 3. ANTIVIRALS - progress and proposals

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter</th>
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</thead>
<tbody>
<tr>
<td>14:40</td>
<td>Report on the variola virus collection at the WHO Collaborating Centre for Smallpox and Other Poxviruses – Centers for Disease Control, USA</td>
<td>Dr V. Olson, Chief, Poxvirus and Rabies Branch, Centres for Disease Control, Atlanta, Georgia, USA</td>
</tr>
<tr>
<td>14:50</td>
<td>Discussion</td>
<td>All</td>
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**Break 10 minutes**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 3. ANTIVIRALS - progress and proposals</th>
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<tbody>
<tr>
<td>15:10</td>
<td>Clinical trials on the anti-smallpox drug NIOCH-14</td>
</tr>
<tr>
<td></td>
<td>Professor S. Shchelkunov, Principal Researcher, Department of Genomic Studies, VECTOR</td>
</tr>
<tr>
<td>15:25</td>
<td>Discussion on VECTOR antivirals research projects</td>
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<td></td>
<td>All</td>
</tr>
<tr>
<td>15:35</td>
<td>Progress update on approved projects</td>
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<tr>
<td></td>
<td>Use of live variola virus to characterize effectiveness of</td>
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<tr>
<td></td>
<td>- antiviral therapeutic tecovirimat (additional data) and</td>
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<tr>
<td></td>
<td>- novel antiviral therapeutic ST-357</td>
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<tr>
<td></td>
<td>Approved 2019/2020</td>
</tr>
<tr>
<td></td>
<td>Dr C. Hutson, CDC</td>
</tr>
<tr>
<td>15:50</td>
<td>Tecovirimat and ST-357</td>
</tr>
<tr>
<td></td>
<td>– research, licensing &amp; production update</td>
</tr>
<tr>
<td></td>
<td>Dr D. Hruby, SIGA Technologies</td>
</tr>
<tr>
<td>16:00</td>
<td>Brincidofovir</td>
</tr>
<tr>
<td></td>
<td>– research, licensing &amp; production update</td>
</tr>
<tr>
<td></td>
<td>Dr O. Naderer, Chimerix</td>
</tr>
<tr>
<td>16:10</td>
<td>Use of live variola virus to evaluate antiviral agents (monoclonal antibodies) against variola virus</td>
</tr>
<tr>
<td>16:20</td>
<td>Use of live variola virus to determine whether mice are a suitable animal model for human smallpox</td>
</tr>
<tr>
<td></td>
<td>Partially approved 2019/2020; Proposed 2020/2021</td>
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<tr>
<td>16:30</td>
<td>Discussion on CDC antivirals research projects</td>
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<td></td>
<td>All</td>
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<tr>
<td>17:00</td>
<td>CLOSE OF DAY ONE</td>
</tr>
</tbody>
</table>
**Thursday, 05 November 2020 – Day two - Dr David Ulaeto, Vice-Chair, ACVVR22**

*Video link: https://who-e.zoom.us/j/94572295202*

### Session 4. VACCINES - progress and proposals

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:30</td>
<td>Recap of discussions and recommendations of Day 1</td>
<td>Dr. R. Drillien, Chair</td>
</tr>
<tr>
<td>13:45</td>
<td>Clinical trials of the anti-smallpox vaccine VACdelta6* (*proposals for use of VARV approved and deferred to 2021)</td>
<td>Dr R. Maksuytov, VECTOR</td>
</tr>
<tr>
<td>13:55</td>
<td>Discussion on VECTOR vaccine research</td>
<td>All</td>
</tr>
<tr>
<td>14:10</td>
<td>Study of MVA-BN vaccine in health workers in Tshuapa Province, the Democratic Republic of the Congo</td>
<td>Dr C. Hutson, CDC</td>
</tr>
<tr>
<td>14:20</td>
<td>LC16m8 smallpox vaccine – research, licensing and production update</td>
<td>Dr Y. Shinmura, KM Biologics, Japan</td>
</tr>
<tr>
<td>14:30</td>
<td>MVA-BN vaccinia vaccine – research, licensing and production update</td>
<td>Dr H. Weidenthaler, Bavarian Nordic</td>
</tr>
<tr>
<td>14:40</td>
<td>Discussion on CDC vaccine research updates and plans</td>
<td>All</td>
</tr>
</tbody>
</table>

**Break 10 minutes**

### Session 5. DIAGNOSTICS - progress and proposals

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:00</td>
<td>Point-of-care rapid diagnostics of orthopoxvirus infections in humans New research proposed 2020/2021</td>
<td>Professor S. Shchelkunov, VECTOR</td>
</tr>
<tr>
<td>15:30</td>
<td>Use of live variola virus to – maintain and regenerate non-infectious variola virus derived materials for diagnostic development support Approved 2019/2020; Proposed 2020/2021 and – develop protein based diagnostic and detection assays specific for variola virus Approved 2019/2020</td>
<td>Dr V. Olson, CDC</td>
</tr>
<tr>
<td>15:45</td>
<td>Discussion of diagnostic research updates and plans</td>
<td>All</td>
</tr>
</tbody>
</table>

### Session 6. Closed session for ACVVR members and advisers

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:00</td>
<td>Discussion on live variola virus research agenda 2020–2022, recommendations and pending issues</td>
<td>All</td>
</tr>
<tr>
<td>17:00</td>
<td>CLOSE OF DAY TWO and ACVVR22</td>
<td></td>
</tr>
</tbody>
</table>
Annex 2  |  Participants

MEMBERS

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

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

Dr Robert Drillien (Chair, day 1), Research Scientist, Institute of Genetics and Molecular and Cellular Biology (IGBMC), Illkirch, France

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

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

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

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 Oyewale Tomori, Professor of Virology and Fellow, Nigerian Academy of Science, University of Lagos, Lagos, Nigeria
Dr Aissatou Toure, Head of Immunology Unit, Institut Pasteur de Dakar, Dakar, Senegal

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

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Professor Hermann Meyer, Head of Department, Bundeswehr Institute of Microbiology, Munich, Germany

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

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

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Annex 3 | Research proposals for 2021

Annex 3. Research proposals presented for 2021 by VECTOR, and WHO approval status

<table>
<thead>
<tr>
<th>Proponent and projects</th>
<th>Yes</th>
<th>No</th>
<th>Majority opinion and notes</th>
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<td><strong>VECTOR</strong></td>
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<td>Use of live variola virus to:</td>
<td></td>
<td></td>
<td>Recommendation of the 22nd ACVVR</td>
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<tr>
<td>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]</td>
<td></td>
<td></td>
<td>Continuation *</td>
<td>November 2019</td>
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<td>2. Discover and test novel chemical antivirals for smallpox treatment and prevention [continuing]</td>
<td></td>
<td></td>
<td>Continuation *</td>
<td>November 2019</td>
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<tr>
<td>3. Evaluate antivirals against smallpox based on monoclonal antibodies [continuing]</td>
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<td></td>
<td>Continuation *</td>
<td>November 2019</td>
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<td>4. Replenish the stocks with non-infectious material, derived from live virus, required for diagnostics development [recurrent]</td>
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<td>5. Develop advanced methods for rapid (point-of-care) diagnostics of orthopoxvirus infections [new]</td>
<td>6 (+1 conditional)</td>
<td>2</td>
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*delays occurred in 2020 due to the COVID-19 pandemic
Annex 3b. Research proposals presented for 2021 by CDC, and WHO approval status

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<td></td>
<td>Recommendation of the 22nd ACVVR</td>
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<tr>
<td>1. Maintain and regenerate non-infectious variola virus derived materials for diagnostic development support [recurrent]</td>
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<td>2. Characterize effectiveness of antiviral therapeutic [tecovirimat] [completing]</td>
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<td></td>
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<td>3. Characterize effectiveness of novel antiviral therapeutic ST-357 [continuing]</td>
<td></td>
<td></td>
<td>Continuation *</td>
<td>December 2019</td>
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<td>4. Evaluate antivirals (monoclonal biologics) against variola virus [continuing]</td>
<td></td>
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<td>5. Determine whether mice are a suitable animal model for human smallpox, providing means to evaluate medical countermeasures against authentic agent [resubmission]</td>
<td>9</td>
<td>1</td>
<td>Yes</td>
<td>Approval November 2020</td>
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<td>6. Support less reactogenic vaccine development: continued evaluation of third generation vaccines [continuing]</td>
<td></td>
<td></td>
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<tr>
<td>7. Develop protein-based diagnostic and detection assay specific for variola virus [continuing]</td>
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<td>Continuation *</td>
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*delays occurred in 2020 due to the COVID-19 pandemic
Annex 4  |  Abstracts of presentations

The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention Atlanta, GA: Report on the variola collection for the 2020 ACVVR meeting

Christina Hutson, Ashley Kondas, Matthew Mauldin, Kimberly Wilkins, Todd Smith, Yu Li, Jinxin Gao, Hui Zhao, Christine Hughes, Victoria Olson
Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA

The World Health Organization (WHO) Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention in Atlanta, GA, continues to maintain one of two consolidated, international collections of variola virus strains. The majority of these viruses were originally isolated on embryonated eggs and characterized during the final years of the intensification of the smallpox eradication campaign. The virus collection is maintained in two separate freezers, one of which is a back-up freezer remaining largely untouched. Secure databases addressing WHO recommendations and USA Federal Select Agent Program requirements have been constructed and maintained to track usage of variola virus. Annual reports on the status of these collections are provided to WHO. No new variola virus seed pools were added to working stocks between 2019 and 2020. In 2020, aliquots from twenty-one original isolates were removed from the repository freezer and twenty were processed for full genomic sequencing (one isolate was not processed as it was determined to be a duplicate and was moved back into the repository without processing). These isolates were selected based on historic information available such as geographic location, date, and epidemiologic data. Since the twenty-first ACVVR meeting, WHO-approved research activities utilizing variola virus from the inventory have focused on: 1) evaluation of monoclonal antivirals (monoclonal biologics); 2) evaluation of a new anti-viral therapeutic (ST-357); 3) regeneration of non-infectious material for diagnostic support; and 4) determining whether humanized mice are a suitable animal model for human smallpox. The laboratory space was in active use from 6 June 2019 through 30 March 2020; the laboratory underwent decontamination prior to preventative maintenance during April 2020. Due to the SARS-CoV-2 pandemic, the laboratory remained in a “cold” state for an extended period and did not become operational again until 24 July 2020. During August 2020, the Federal Select Agent Program conducted a virtual inspection and had no findings. In the United States, variola virus is a select agent and is subject to the select agent regulations (42 CFR part 73).
Report on the variola virus collection at the WHO Collaborating Center for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at FBRI SRC VB VECTOR, Rospotrebnadzor

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

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

Currently, the VARV collection comprises 120 strains, originating from Europe (EURO), Asia (SEARO), Africa (AFRO), South America (AMRO), and the Middle East (EMRO).

The VARV strains in the repository are stored in a freeze-dried or frozen form as well as in the form of primary specimens isolated from human patients in the past (scabs).

The work with live variola virus is scheduled to be resumed early in 2021 following the WHO approval of the VECTOR-proposed research projects involving the use of live variola virus:

- Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development.
- 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.
- Discovery and testing of novel chemical antivirals for smallpox treatment and prevention.
- Use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies.
- Development of advanced methods for rapid (point-of-care) diagnostics of orthopoxvirus infections.
Use of live variola virus to characterize effectiveness of anti-viral therapeutic TPOXX

Christina Hutson, Todd Smith, Matthew Mauldin, Christine Hughes, Ashley Kondas, Jinxin Gao, Yu Li, Victoria Olson

Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

Douglas W. Grosenbach, Dennis E. Hruby, Andrew Russo, Lek Chinsangaram, Candace Lovejoy

SIGA Technologies, Inc.

Current recommendations support the need for at least two antiviral compounds, with discrete mechanisms of action, to be licensed and available should smallpox reemerge. The US Food and Drug Administration (FDA)-approved therapeutic agent tecovirimat (ST-246 or TPOXX) has been tested extensively in vivo within multiple animal models, including the non-human primate variola virus (VARV) model. In the United States, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73). Although tecovirimat has shown efficacy against multiple orthopoxviruses, it has also been noted that changes in the orthopoxviral F13L gene allows for resistance to tecovirimat; additionally, resistance emerged during use of the drug in an extended treatment course of an individual with progressive vaccinia. Therefore, 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 (AA) variants in vitro against tecovirimat. Using NCBI's BLAST algorithm, 53 published F13L genes were identified from GenBank. These, along with 61 sequences from unpublished genomes recently generated by CDC (total n=114) were compared by visual examination of multi-sequence alignment and haplotype analyses. Fifteen nucleotide haplotypes (unique sequences) were identified which translated to 8 unique AA variants, with one of these unique variants identified only within the new unpublished CDC sequence data. Within the CDC repository, isolates from 6 of the 8 variants were available for screening. Variant 1 contains 78.1% of all isolates (94.7% of all isolates belong to one of three AA variants [1, 5, or 7]). Variants 3, 4, 6 and 8 contain only one isolate each. Multiple isolates from variants 1 and 5 were tested previously. Variant 6 was identified from an isolate only available at the VECTOR repository, which has also been previously tested with a compound of similar structure to tecovirimat. Variant 8 was identified from one CDC unpublished sequence; however, the sample was used to completion for sequencing so was not available.

As detailed at the 2019 ACVVR meeting, using a cytopathic effect assay, 7 VARV isolates from 5 AA variants were screened for tecovirimat sensitivity. Results showed all isolates were sensitive with half-maximal effective concentrations (EC50) of 0.01 – 0.03 µM. The 90% effective concentration (EC90) was also in the nanomolar range (0.02 – 0.15 µM) for all isolates. Although isolates from variant 1 had been previously screened, Japan_1951_Harper isolate was selected from this variant due to its use in in vivo models and for comparison to previous results. Indeed, the EC50 for Japan_1951_Harper isolate was similar to published EC50 data for previously screened variant 1 isolates. These results increase confidence in the effectiveness of tecovirimat as a medical countermeasure and help in fulfilling the post-marketing commitment with FDA.
However, we are continuing to expand our genetic database of VARV sequences and have identified additional F13L AA variants within original VARV samples; additional variants may be identified as CDC finalizes sequence results. Including all newly sequenced VARV isolates, there are now 18 unique nucleotide haplotypes, which translate to 10 unique AA variants. Using BLAST, 180 VARV F13L gene sequences were compared. For isolates that are unavailable for tecovirimat sensitivity testing, we will utilize 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 (IBC 2013.251/252). This approach avoids creating a recombinant virus that contains VARV sequences. We have obtained the required institutional approvals to generate stable cell lines expressing different VARV F13L protein variants (IBC 2019.394). Cloning and site-directed mutagenesis of the required genes are in progress and results/study plans will be shared at the November 2020 ACVVR meeting.
Use of live variola virus to characterize effectiveness of novel anti-viral therapeutic ST-357

Christina Hutson, Todd Smith, Matthew Mauldin, Christine Hughes, Ashley Kondas, Jinxin Gao, Yu Li, Victoria Olson
Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

Douglas W. Grosenbach, Dennis E. Hruby, Andrew Russo, Lek Chinsangaram, Candace Lovejoy, Jim Burgeson Biswajit Maiti
SIGA Technologies, Inc.

The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox somehow re-emerges. This includes the development of antiviral strategies. In July 2018, the antiviral tecovirimat (or ST-246) was licensed by FDA for smallpox treatment. Current recommendations recognize the need for at least two antiviral compounds, with discrete mechanisms of action, to be licensed and available for use. Additionally, since pivotal clinical trials cannot be conducted in humans (because the disease is eradicated), multiple medical countermeasures are required in case of failure of one. As demonstrated by a recent clinical trial against Ebola virus disease, even highly effective therapeutics in the non-human primate model can fail in human clinical trials. Currently, another antiviral compound (ST-357 or TTP-018) has been identified targeting viral, mRNA poly-A polymerase encoded by E1L, a different target than tecovirimat. A slight synergistic antiviral effect was observed when cells were treated with both tecovirimat and ST-357. The parental ST-357 drug has been shown to be effective in vivo against lethal ectromelia, cowpox and vaccinia virus challenge studies in mice. Medicinal chemistry and analogue screening are under way. If a preclinical candidate can be identified and found efficacious against variola virus (VARV), this would supplement the anti-viral medical countermeasure arsenal, better preparing the global community should a release of smallpox occur. During the first year of proposal approval (first approved December 2019), the parental compound ST-357 was screened against live VARV. A total of three isolates were screened: VARV Bangladesh_1974_Solaiman, Sierra Leone_1969_V68-258, and Japan_1951_Harper. All three of the isolates were sensitive to ST-357 with half-maximal effective concentrations (EC50) of 0.04 – 0.05 µM. Since ST-357 inhibited VARV growth in vitro, preclinical candidates from the ST-357 analogue series were screened against vaccinia virus (VACV). In the future, as candidates are shown to be effective against VACV, they will then be screened against representative VARV isolates. During the previous year, two ST-357 analogues were screened using VACV Western Reserve with EC50 of 0.14 – 0.31 µM compared to 0.055 µM for the parental compound. Currently, CDC is awaiting additional preclinical candidates from SIGA. This has been delayed due to the COVID-19 pandemic. Assuming a preclinical candidate meets all necessary criteria (e.g. bioavailability, solubility, stability, and antiviral activity) following medicinal development and in vitro testing, as the lead compound moves through final production phases, additional screens against VARV in vitro with the cytopathic effect (CPE) assay will likely need to be performed. The results from the previous year and planned work during 2021 will be presented at the ACVVR meeting in November 2020. In the United States, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73).
Preclinical studies and clinical trials on the anti-smallpox drug NIOCH-14

Shchelkunov SN, Shishkina LN, Bormotov NI, Skarnovich MO, Sergeev AIA, Skarnovich MA, Mazurkov OYu, Serova OA, Gamaley SG, Shimina GG, Sysoyeva GM, Bateneva AV, Taranov OS, Danilenko ED, Bogryantseva MP, Agafonov AP, Maksyutov RA

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

A finished dosage formulation (FDF) of the anti-smallpox drug, NIOCH-14, has been developed in the form of hard gelatin capsules for oral use.

The FDF formulation of NIOCH-14 is low toxic since when administered intragastrically at a dose of 5 g/kg it did not cause the death of mice, and it is safe as judged by the values of acute and chronic toxicity in experiments in mice and rats. With both a single and multiple daily (for 30 days) intragastric administration of NIOCH-14 FDF at doses of 50 and 150 mg/kg of weight in mice and rats, no pronounced or long-lasting changes in physiological, histological, haematological or biochemical parameters were observed. The NIOCH-14 FDF did not reveal any immunotoxic or allergenic properties, reproductive toxicity or mutagenic activity in mice, rats, or guinea pigs.

In solvents used for mass spectrometry, and in the bodies of animals, NIOCH-14 is converted into an active metabolite ST-246. Following a single oral administration to mice of a suspension of NIOCH-14 FDF in a capsule at a dose of 50 μg/g of body weight, the maximum concentration (Cmax) of ST-246 in the sera of mice reached 19.63 μg/ml within 3 hours while the absolute bioavailability (Fabs) made 61.1%. Following a single oral administration to rabbits of NIOCH-14 FDF in a capsule at a dose of 60 μg/g of rabbit body weight, the Cmax value of ST-246 was 0.63 μg/ml within 6 hours while the Fabs value of NIOCH-14 FDF in rabbits made 5.0%.

In the intranasal infection of mice with ectromelia virus (ECTV) at a dose of 10 LD50 and the administration of NIOCH-14 FDF, its 50% effective dose was 4.12 μg/g of mouse body weight, and when using doses greater than 12.5 μg/g, the mouse survival rate was 100%. The therapeutic window, a period of effective use of NIOCH-14 FDF, reached 5 days post ECTV infection; the median survival time increased truly, and the virus titres in the lungs, liver and spleen decreased in relation to the corresponding values in a control group of mice infected with ECTV.

In the groups of mice that were infected with variola virus (VARV) at a dose of 30 ID50 and received NIOCH-14 FDF, the percentage of infected and virus titres in the lungs truly decreased compared to the corresponding values in a control group of mice infected with VARV.

It was demonstrated that NIOCH-14 FDF was safe, bioavailable, and highly effective against orthopoxviruses when administered orally.

On 22 July 2020, authorization was granted by the Ministry of Health of the Russian Federation to conduct Phase I clinical trials on the drug NIOCH-14. On 4 September 2020, clinical trials of NIOCH-14 were started that involved 90 volunteers (aged 18 to 50 years old) and that will last for 6 months.
**Tecovirimat and ST-357 – research, licensing and production update**

_Dennis E. Hruby, Doug Grosenbach, Andrew Russo, Jaravesch Chinsangaram, Kady Honeychurch, Candace Lovejoy, Paul Long, Biswajit Maiti_  
_SIGA Technologies, Inc., Corvallis Oregon, United States of America_  

_Christina Hutson, Todd Smith, Matthew Mauldin, Christine Hughes, Ashley Kondas, Jinxin Gao, Yu Li, Victoria Olson_  
_Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States of America_

ST-246/tecovirimat oral capsule formulation (TPOXX®) was approved by the FDA in July of 2018 for the treatment of symptomatic smallpox in adult and paediatric patients weighing ≥13 kg. At the time of approval, FDA issued a post-marketing requirement to “Conduct a field study to evaluate the clinical response, drug concentrations, and safety profile of tecovirimat when used in the treatment of subjects with variola virus infection”. The study protocol has been finalized and approved by FDA and will be deployed in the event of a smallpox outbreak in the US. Additional post-marketing commitments were requested by FDA and included 1) evaluation of numerous vaccinia and variola isolates with nucleotide polymorphisms in the F13L or C17L gene (respectively) for susceptibility to tecovirimat; 2) conduct a study to determine the pharmacokinetics of tecovirimat in subjects with body weight greater than 120 kilograms; 3) conduct an in vitro study to determine the potential for a drug interaction between tecovirimat and phosphate binders; and 4) conduct and submit a risk assessment for elemental impurities when new batches of the drug product are manufactured. Several of these commitments have been fulfilled while others are in process or delayed due to COVID-19. To date, SIGA has supplied the US Strategic National Stockpile with more than two million courses of therapy and continues to re-supply as product expires.

SIGA has recently been engaged by the Canadian government to supply tecovirimat to the Public Health Agency and the Department of National Defense. In support of this effort, SIGA anticipates filing a New Drug Submission in Canada in Q4 2020. SIGA has already filed a Marketing Authorization Application with the European Medicines Agency. Both the Canadian and European filings seek approval for treatment of human orthopox disease without restriction for the smallpox indication only. Meanwhile, tecovirimat continues to be available for emergency use to treat orthopoxvirus infections via E-IND applications in the US and compassionate use or named patient use outside the US. SIGA is working with Oxford University to develop a clinical protocol for an observational study to treat monkeypox patients in Africa with tecovirimat. SIGA continues to advance the tecovirimat intravenous formulation towards approval (NDA submission anticipated Q1 2021) as well as a powder for reconstitution formulations (PfR) for smallpox patients unable to swallow an oral capsule (e.g. extremely ill/dysphagic or paediatric patients <13 kg).

There remains a health security gap between the time point that post-exposure vaccination is effective (~ day 4 post-exposure) and when smallpox becomes symptomatic and tecovirimat is then indicated (as late as 17 days post-exposure). This gap can be bridged by gaining approval of the post-exposure prophylaxis (PEP) indication for tecovirimat. SIGA is currently partnering with the US DoD to develop tecovirimat for the PEP indication. Following a Type C meeting, the FDA has
indicated that no additional animal studies would be necessary for a PEP approval, but that additional safety and evaluation of the impact of tecovirimat on MVA-BN\textsuperscript{25} immunogenicity would have to be studied in human clinical trials. These clinical trial protocols have been developed and are currently under review by the FDA.

Finally, SIGA is developing a second smallpox antiviral that targets a mechanism of action distinct from tecovirimat (as well as other poxvirus therapeutics in development). A second antiviral could be used as either standalone or in combination therapy or as a second-line therapy for the treatment of resistant viruses. There is also the potential for a more broad poxvirus indication as the target is conserved between orthopoxviruses, molluscipoxviruses and even cervidpoxviruses. This would satisfy a requirement from the US government for a second option for smallpox treatment.

\textsuperscript{25} A non-replicating vaccinia vaccine, renamed as IMVANEX® in the European Union, as IMVAMUNE® in Canada, and as JYNNEOS® in the United States.
Brincidofovir development update

Odin Naderer
Chimerix, Inc.

Introduction:
Smallpox remains a national security threat in the United States and a global public safety concern due to its potential as a bioterror weapon and the possibility of an accidental release. Recent developments in synthetic biology make de novo synthesis of resistant orthopoxviruses a potential concern. This has heightened the need for effective antiviral agents with different mechanisms of action and/or high barriers to drug resistance. One such agent is brincidofovir (BCV), an orally bioavailable lipid conjugate of cidofovir (CDV) that was designed to enhance the intracellular concentrations of the active antiviral, cidofovir diphosphate (CDV-PP). A comprehensive development programme in concordance with the FDA’s Animal Rule has been completed for BCV in the treatment of smallpox. Regulatory activities for BCV are ongoing and Chimerix expects potential approval in 2021. This abstract describes the multi-stage modelling and simulation approach to determine the BCV dose for the treatment of smallpox in humans.

Methods:
BALB/c mouse and New Zealand white (NZW) rabbit plasma BCV and intracellular (PBMC) CDV-PP pharmacokinetics (PK) were obtained from multiple infected and uninfected animal studies. Mouse sampling was volume limited and required multiple animals to create single composite timepoint assessments of plasma BCV and CDV-PP concentration. Robust sampling was feasible in the rabbit and allowed for the development of a rabbit population PK (POPPK) model. A pooled survival analysis model was developed using efficacy data obtained from three studies in NZW rabbits infected with a lethal inoculum of rabbitpox. Human plasma BCV and CDV-PP data obtained from healthy subjects were integrated into a POPPK model, which allowed for simulations of various human dose regimens. Healthy human exposures were used as reference for smallpox patients and represent the most conservative exposure measure in humans. The BCV and CDV-PP concentration–time profiles in rabbit and healthy volunteers were described using a parent–metabolite POPPK model. Comparison of the simulated BCV and CDV-PP PK in healthy humans were made to the efficacious exposures in rabbits to select the clinical dose. Direct comparisons of the rabbit and human concentration–time profiles were adjusted to account for the orthopoxvirus disease course differences between species to provide an accurate comparison of the adequacy of the human dose regimen.

Results:
Survival analyses in the rabbitpox model identified BCV dose, treatment initiation day, and baseline viral load at treatment as significant predictors of survival, with baseline viral load selected as an important covariate in the survival modelling. The results showed survival benefit at a total BCV dose of 20 mg/kg, which plateaued when the total dose was ≥ 30 mg/kg (i.e. ≥ 20/5/5 mg/kg q48 hours). The POPPK rabbit model was then used to simulate the BCV and CDV-PP PK in healthy and infected rabbits at the efficacious 20/5/5mg/kg regimen. In humans, the mean plasma BCV Cmax and AUC following the first 200 mg dose were approximately 2-fold higher than the plasma BCV exposures simulated in healthy rabbits following the 20 mg/kg dose in the 20/5/5 mg/
kg dose regimen. The CDV-PP Cmax and AUC were 2- and 8-fold higher in humans. Similar BCV and CDV-PP Cmax and AUC ratios were observed between healthy adults and infected rabbits. The human to rabbit BCV and CDV-PP exposure margin increased following the second dose in humans due to the decrease in the BCV dose administered to rabbits (5 mg/kg) and accumulation of CDV-PP in PBMCs in human. Human CDV-PP exposures were similar to mouse exposures at the efficacious dose of 10/5/5 mg/kg.

Conclusions:
In summary, the analyses demonstrated that the proposed BCV dose of 200 mg once-weekly given on day 1 and day 8 delivered BCV and CDV-PP mean concentration profiles that were above the efficacious exposures observed in healthy and rabbitpox infected rabbits receiving the 20/5/5 mg/kg q48h regimen for the duration of the smallpox disease course. The analyses also showed that the human exposures were similar to exposure at the efficacious treatment in the mousepox model. These results support the clinical dose recommendation in humans.
Use of live variola virus to evaluate monoclonal biologics against variola virus

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The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox re-emerges. In the United States, variola virus (VARV) is a select agent and subject to select agent regulations (42 CFR part 73). Therapeutic strategies are important for outbreak response efforts as well as disease treatment. Current considerations have suggested the need for at least two therapeutic compounds with discrete mechanisms of action to be licensed and available for use. Recently, tecovirimat was the first therapeutic to be licensed by the US Food and Drug Administration (FDA) for treatment of smallpox. However, the search for the second therapeutic continues; monoclonal antibodies (mAbs) or cocktails could be an option to fill this need. In recent years several mAbs have received FDA approval for treatment of multiple conditions/pathogens, including cancer, anthrax, and respiratory syncytial virus. The Biomedical Advanced Research and Development Authority (BARDA) has recently announced an R&D contract for the advanced development of a potential medical countermeasure (MCM) for the treatment of smallpox. A mAb cocktail for orthopoxviruses (OPXVs) could be particularly beneficial as it would include at least two mAbs to target both infectious forms of virus, potentially limiting likelihood of viral resistance by having multiple viral targets.

In 2020, CDC efforts focused on identifying biologic therapeutics against VARV that were previously shown to neutralize other OPXV (both in vitro and in vivo) utilizing our plaque reduction neutralization assays (PRNT). We have continued our collaboration with the Vanderbilt Vaccine Center and identified 3 VARV intracellular mature virion (IMV) neutralizers and 7 VARV external enveloped virion (EV) neutralizers. Previously, the Vanderbilt Vaccine Center created two cocktails of mAbs (Mix4 and Mix6) with high capacity to neutralize both forms of infectious virus across multiple non-VARV OPXVs. However, new VARV data suggest that neither Mix is ideal for VARV treatment as the EV targeted mAbs are not strong at neutralizing VARV or monkeypox virus (MPXV). In 2019, we completed re-evaluation of mAbs that neutralized VARV against MPXV to develop a Universal OPXV Mix. This presentation will include these findings as well as plans to develop the Universal OPXV Mix.

Commercial entities have also begun production of mAbs directed against OPXVs. In 2018, we presented preliminary data of individual mAbs and cocktails from 2 commercial entities (Company A and Company B) to neutralize VARV. In 2019, cocktails from 3 commercial entities underwent evaluation utilizing the intravenous non-human primate (NHP) model where treatment was administered post exposure following a lethal MPXV challenge. Survival rates from Company A and B were 9/10 and 8/10, respectively. In parallel to the NHP study, CDC determined EC50 values for individual mAbs and cocktails against both IMV and EV forms of VARV using our PRNT assays for Company A and Company B. Our data demonstrate that Company A and Company B cocktails neutralized VARV in
vitro. In late 2019, CDC received the individual mAbs and cocktail from company C and evaluation was completed in 2020. Here we will provide additional information on the VARV EC50 values determined in our IMV and EV PRNTs, an update on the humanization of the mAbs, bioinformatic analysis of epitope targets, and future steps towards licensure including humanization of the mAbs as well as final quality control testing.
Use of live Variola virus to determine whether humanized mice are a suitable animal model for human smallpox

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In the United States, variola virus (VARV) is a select agent and is subject to the select agent regulations (42 CFR part 73). Historically, laboratory research efforts have tested several animal species for susceptibility to VARV, but as of yet, non-human primates (NHPs) are the only non-human animals which exhibit overt illness. In order to induce illness in NHPs, the required infectious dose (~1x10^8 VARV plaque forming units [pfu]) is much greater than the dose required for a natural human smallpox infection, and the model necessitates an intravenous inoculation. Because of these limitations, a number of surrogate animal models of orthopoxvirus (OPXV) disease have been developed to evaluate the efficacy of various safer smallpox vaccines/therapeutics. Many of these models also have limitations, such as short disease incubation periods, which do not resemble human smallpox. As a result, these systems are suboptimal for evaluating efficacy of antivirals as therapeutics (after symptom onset). The development of a more permissive/representative VARV animal model would facilitate testing of therapeutics.

Humanized mice (hu-mice) have become an invaluable tool for modelling human disease and investigating human-specific therapeutic candidates and provide an excellent alternative to NHPs.

Previously, we presented our 2015 pilot study which evaluated 3 types of humanized female mice (BLT, hu-CD34 and PBMC), obtained from Jackson Laboratories. During this pilot study, mice were inoculated via an intranasal (IN) challenge with VARV (7x10^3 or 7x10^5 pfu JAP51_hrpr strain). This study identified that following viral challenge, all three mouse strains were susceptible to VARV infection, and subsequent pathologic, electron microscopy, tissue culture and immunohistochemical (IHC) analyses confirmed infection as the cause of mortality in mice that succumbed to disease following an approximate 13-day window. We further characterized the model by performing a dosage and serial sacrifice studies to characterize viral trafficking. The features of this animal model make it suitable for efficacy testing of potential antiviral therapeutics against VARV infection; i.e. in the ability of therapeutics to protect mice following a VARV challenge. The BLT serial sacrifice study showed that the infection is systemic by day 9 pi, which is approximately 3–4 days before mortality; this time frame would allow for rigorous therapeutic testing in this animal model. In 2020, we have processed samples for molecular assays and are working on data analysis to finalize viral trafficking, biomarker evaluation and seroconversion.

Having these models fully characterized, and results published in a peer-reviewed journal, will allow the FDA to assess the usefulness of this animal model for evaluation of smallpox medical countermeasures. FDA does not require additional evaluation of ST-246 (tecovirimat) against live VARV in vivo but may require this information should there be an attempt to gain licensure for another anti-VARV therapeutic. By characterizing this unique animal model, the global community will have vital information concerning its utility for testing future therapeutics against VARV in vivo. At the November 2020
ACVVR we will update progress with model characterization as well as detail our proposal to conduct two efficacy studies with ST-246 treatment to validate if this animal model is relevant for evaluation of new anti-viral therapeutics. ST-246 is the only currently FDA-approved therapeutic against smallpox and thereby serves as an excellent control to confirm if the hu-mouse VARV model accurately evaluates the efficacy of smallpox antiviral agents. If we are allowed to proceed with these ST-246 studies and the results validate the hu-mouse VARV model, it could be used to test the efficacy of brincidofovir in vivo (currently being developed for the treatment of smallpox under the US Food and Drug Administration’s Animal Efficacy Rule). Such a study would be extremely useful as, unlike ST-246, brincidofovir could not be tested with any NHP models (VARV or MPXV) due to rapid drug metabolism within the NHP model.
Use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines

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Variola virus (VARV) neutralization in vitro remains an informative surrogate measure of smallpox vaccine efficacy. The plaque reduction neutralization test (PRNT), which measures the ability of immune sera to neutralize mature virus forms (MV), has been used as a primary endpoint for the evaluation of vaccines. Our prior studies, using sera from vaccinia virus-vaccinees (MVA-BN\textsuperscript{26}, ACAM2000, Dryvax or LC16m8), have indicated that neutralization endpoint titres may differ when using different species of target viruses. Slight differences in orthopoxvirus antigens likely account for these differences. Development of new vaccines has included significant focus on use of attenuated vaccine strains, such as modified vaccinia Ankara (MVA) and LC16m8. These “third generation” vaccines, however, were never tested directly for efficacy against smallpox during the eradication campaign since most were developed towards the end or after eradication. We have found a statistically significant difference in neutralization titres of vaccinee serum when using different target viruses (VARV - heterologous target versus vaccinia virus - homologous target). Therefore, evaluation of the ability of sera to neutralize the MV form of VARV will provide a more informative surrogate measure of efficacy. The role of VARV neutralization as a surrogate marker for vaccine efficacy is particularly valuable for evaluation of vaccines like MVA that do not elicit a “take”, the traditional measure of vaccine success.

In 2018, results of the VARV PRNT optimization data for analysis of Bavarian Nordic’s MVA-BN were included within the Analytical Plan and Redevelopment Report, which were submitted and approved by the United States Food and Drug Administration (FDA). The study (n=100 per vaccine [50 pre and 50 post taken at “peak” time-point for each vaccine]) final titres were calculated and presented at the 2019 ACVVR meeting. Statistical comparison of average fold-rise 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, will be supplemental material to support the non-inferiority clinical trial dataset. MVA-BN was approved by FDA in September 2019 for prevention of smallpox and monkeypox. During the 2020 approval period, raw data (well images and plaque counts) of VARV PRNT assay runs were organized and archived for future availability/transparency. Additionally, manuscripts were prepared to make these data publicly available.

CDC has been involved in an MVA-BN vaccine trial in the Democratic Republic of the Congo where monkeypox virus (MPXV) remains endemic. The COVID-19 pandemic has slightly delayed testing of study serum samples. All serum from ~1000 participants in the

\textsuperscript{26} A non-replicating vaccinia vaccine, recently renamed as JYNNEOS® in the United States.
Democratic Republic of the Congo MVA-BN vaccine study (liquid formulation, part 1 of the study which included time points of day 0, 14, 28, 42, 6 months, 1 yr, 1.5 yr, and 2 yr) have been tested by IgG and IgM ELISA (against vaccinia virus), with data cleaning and analysis ongoing. Neutralization testing against vaccinia virus began in September and has proceeded as time permitted. Part 2 of the study with a lyophilized formulation of MVA-BN vaccine has continued through the 6-month time point; however, the 1-year study visit was cancelled due to the COVID-19 pandemic. We are addressing safety concerns in the hope of collecting the 1.5-year visit in February.

As serum sample collection continues from the Democratic Republic of the Congo trial, neutralization against VARV in vitro can be assessed at additional time points beyond peak titre. Understanding the longevity of humoral immunity will allow for efficient use of stockpiles, and development of effective utilization strategies, allotting more doses for protection of those most at risk. Valuable insights into longevity of immune response can be gained by screening samples for ability to neutralize VARV since differences in neutralization capacity have been documented dependent on the orthopoxvirus species used as target within the PRNT assay. In the USA, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73).
Clinical trials of the VACΔ6 vaccine against orthopoxvirus infections

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The smallpox vaccine, VACΔ6, was created based on the vaccinia virus LIVP strain by the alteration of six virulence genes encoding hemagglutinin (A56R), γ-interferon binding protein (B8R), thymidine kinase (J2R), complement-binding protein (C3L), Bcl2-like inhibitor of apoptosis (N1L), and an inhibitor of antigen presentation (A35R) by major histocompatibility complex (class II).

In the course of the vaccine’s preclinical studies, the specific activity and safety of the developed prophylactic product was demonstrated. In 2019, Phase I clinical studies were initiated that involved 60 volunteers. The objective of the studies was to examine the safety, tolerability and immunogenicity of the fourth generation smallpox vaccine VACΔ6 when administered intracutaneously.

It was demonstrated that local skin reactions with the intracutaneous administration of the VACΔ6 vaccine, both in single and double administration, in most cases were characterized by a weak development of post-vaccination elements (hyperaemia, papule, vesicle, and pustule). Symptoms of general reactions such as weakness, headache, and fever were not reported during the entire observation period. The use of the VACΔ6 vaccine is associated with the generation of specific antibodies to orthopoxviruses in the sera of volunteers, regardless of the dose and regimen. When using the reference product (the first generation vaccine used in the Russian Federation), malaise, headache, fever, sometimes up to 38–39°C, and local lymphadenitis were reported.

Based on the results of Phase I clinical studies, authorization of the Ministry of Health of the Russian Federation No. 380 dated 30 July 2020 was granted to conduct Phase II–III clinical studies on the smallpox VACΔ6 vaccine in 334 volunteers. This study consists of two stages. The first stage is a randomized open comparative study in parallel groups. At this stage, a study of the protection effectiveness of the VACΔ6 vaccine will be conducted. The second stage is a double-blind, comparative, randomized, placebo-controlled study in parallel groups. Phase II–III clinical studies are scheduled to begin in November 2020.
A single dose of the MVA-BN smallpox vaccine induces an early protective antibody response and is a vaccine for monkeypox

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Background:
In the Democratic Republic of the Congo 6231 cumulative monkeypox (MPX) cases were reported from week 1 to week 40 in 2020, including 203 fatalities. In addition, over 180 MPX cases have been reported in Nigeria since 2017, with travellers to the UK, Israel, and Singapore becoming ill and incidents of secondary transmission further raising concerns, as growing parts of the population have no pre-existing orthopoxvirus immunity.

The MVA-BN vaccine (JYNNEOS[US], IMVAMUNE[CAN], IMVANEX[EU]) is licensed for both MPX and smallpox (SPX) in the US, with increasing international focus on its use during MPX outbreaks. While past data suggest that traditional, replicating SPX vaccines are 85% effective in preventing MPX, the nonreplicating MVA-BN vaccine can be administered to a wider population, including those with immunodeficiency disorders, with less risk of serious side effects. Nonclinical data strongly support the protective efficacy of MVA-BN against orthopoxviruses, even when administered as a single dose in post exposure settings. Consequently, individuals in England were vaccinated with MVA-BN in response to MPX and a CDC sponsored trial in the Democratic Republic of the Congo is evaluating safety and efficacy of MVA-BN in MPX prevention.

Methods:
A phase 3 noninferiority trial provided data on the onset of protective antibody responses following MVABN and ACAM2000. The coprimary endpoints of this trial were (1) to compare vaccine-induced serum neutralizing antibodies (geometric mean titre [GMT]) at predefined peak visits, as measured by plaque reduction neutralization tests (PRNT) and (2) to assess the attenuation of ACAM2000 induced takes after MVA-BN by measuring maximum lesion area (MLA). Neutralizing antibody GMTs at day 14, a timepoint considered protective for replicating SPX vaccines, were also compared following single doses of either vaccine. 440 subjects were evenly randomized to receive either 2 standard doses of MVA-BN (1x108 TCID50) followed by 1 dose of ACAM2000 at 4-week intervals (Group 1) or a single dose of ACAM2000 (Group 2).

Results:
Peak neutralizing antibody GMTs were significantly higher after MVA-BN (153.5) compared with ACAM2000 (79.3), with a ratio of 1.935 (95% CI: 1.562, 2.397). The median MLA induced by ACAM2000 was significantly reduced when subjects received prior MVABN in group 1 (0 mm2) compared with group 2 (76.0 mm2), demonstrating take attenuation and providing a solid efficacy marker. At day 14, neutralizing antibody GMTs were equal following a single dose of either MVA-BN or ACAM2000 (16.2), with similar seroconversion rates (90.8% vs 91.8%, respectively), demonstrating the suitability of MVABN in both pre- and post-outbreak scenarios. Injection site pain was the most common AE and no MVA-BN-related SAEs or AEs of special interest were reported.
Conclusions:
A single dose of MVA-BN provides an early protective vaccinia-specific antibody response similar to a replicating SPX vaccine, can be safely administered to those with atopic dermatitis and immunodeficiency disorders, and is the only approved MPX vaccine.

Acknowledgement:
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References:
Use of live variola virus to maintain and regenerate non-infectious variola-derived materials for diagnostic development support

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The ability to validate nucleic acid-based and protein-based diagnostic capacity is critical for early detection and recognition of smallpox should a bioterror incident occur. The consequences of either false negatives, or false positives, will significantly impact global public health efforts. As has occurred in the recent past, new isolates of orthopoxvirus are being identified and can confound current diagnostic assays. Furthermore, “older” nucleic acid diagnostic platforms are no longer being supported by some companies, necessitating validation of newer platforms. The need to maintain variola virus (VARV) DNA and VARV antigen stocks at the WHO Collaborating Centre (WHO CC) for Smallpox and other Poxvirus Infections remains important for future diagnostic development and validation. In the United States, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73). Building rapid and accurate diagnostic capacity for use in laboratories worldwide is critical for successful disease containment in the event of smallpox reemergence. Evidence of this occurred during the 2014–2016 Ebola response in West Africa, where the need for rapid and accurate diagnostic capacity in remote or central laboratories was critical for effective disease control. This presentation will update on results from utilization of VARV to validate several DNA diagnostic assays.

Assay validation is substantially more robust when validated with extracted genomic DNA, representative of what would be extracted from a clinical isolate, rather than plasmids expressing the target portions of DNA. For sensitivity analyses, use of virus DNA that has been extracted from purified virions allows a calculation of the limit of detection (LoD). Such materials will continue to be used to validate detection assays from WHO member countries. Additionally, greater understanding of the variability within VARV genomes will be instrumental to understand the sensitivity and specificity of nucleic-based diagnostic assays. During the last year, 20 original VARV samples were processed and sequenced without propagation. Further, VARV samples sequenced in previous years had compilation completed. These new sequences will be utilized to interrogate both CDC diagnostic assays as well as assays our collaborators are developing. Further genomic sequencing has also identified variability in the amino acid sequence of the target for the only approved antiviral compound for smallpox (tecovirimat). Enhancing our database of VARV sequences will greatly improve our understanding of limitations and ability to properly utilize medical countermeasures such as diagnostics and antiviral therapies.

Diagnostic development has continued over the past year, focusing on validations of new reagents and/or equipment as technology advances to retain the US Food and Drug Administration (FDA) approval. The COVID-19 outbreak has highlighted the importance of having multiple options for master mixes and primer and probe manufacturing available for critical real-time PCR assays. Because of this, PRB continues to evaluate different primer/probe chemistries, master mix reagents, and extraction technologies.
(focusing on those platforms most commonly in use within state and local health laboratories) with the FDA approved assays for detection of orthopoxviruses. We also made progress with the portable Oxford Nanopore MinION sequencer. In 2019 we developed a wet lab protocol for sequencing 1–3 kb PCR amplicons and a portable bioinformatics pipeline for data analysis. The pipeline performs de novo assembly of consensus sequences from amplicons and can be run on a laptop running Windows or Linux. We had several successful sequencing runs on a new device (called a Flongle) that uses disposable flow cells, which increases biosafety and biosecurity of sequencing novel poxviruses, monkeypox virus or suspect VARV samples. Finally, we continue to optimize our multiplex, real-time PCR assay for use on the portable nucleic acid amplification test27 in collaboration with BioGX. Studies are focused on optimizing novel delivery methods that minimize manipulations by the technician while retaining sensitivity of the assay. The results of these experiments will be discussed in more detail at the November 2020 ACVVR meeting during the diagnostics presentation along with protein-based diagnostics.

27 GeneXpert® System.
Use of live variola virus to develop virus-specific protein-based diagnostic and detection assays

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The ability to validate protein-based diagnostic capacity is critical for rapid detection and response in case of 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. Alternatively, point of care (POC) assays for antibody or antigen detection hastily adapted during epidemics have the potential to lead to public distrust and hamper global public health efforts if tests are inaccurate and insensitive. These events highlight the two sides of protein-based diagnostic assay development and use. This presentation will update on results from utilization of variola virus (VARV) to validate several protein-based diagnostic assays for accuracy and sensitivity. In the USA, VARV is a select agent and is subject to the select agent regulations (42 CFR part 73).

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 remains the most sensitive for detection of orthopoxviruses (OPXVs) and that incorporation of a mAb with pAb involves trade-offs between sensitivity and specificity. Highly specific VARV mAbs in combination with pAb have shown detection of VARV around $5 \times 10^4$ pfu/ml, but low-level cross-reactivity with other OPXVs was observed with these combinations at concentrations higher than $1 \times 10^7$ pfu/ml. These results suggest a multiplexed assay incorporating mAbs, with varying species specificity, along with pAbs will provide the most robust POC assay.

We previously began collaborations with researchers at Arizona State University (ASU) and with Tetracore. ASU has developed a low-cost multiplex fluorescent lateral flow assay (LFA), which improved the sensitivity by 2–3 orders of magnitude compared to traditional LFA formats. We are evaluating fluorescent LFA using mAbs directed against vaccinia virus or monkeypox virus (MPXV). Based on these results, we will later incorporate VARV detection mAbs in the multiplex assay. We also began exploring other platforms expected to improve antigen detection sensitivity. In collaboration with Tetracore, we utilized a Luminex-based assay and observed a limit of detection as low as $1 \times 10^3$ 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 pending the resolution of the COVID-19 pandemic.

We have continued evaluating the utilization of LFA for field use in Democratic Republic of the Congo, which is endemic to MPXV infections. We have begun enrolment for this OPXV generic POC test to detect MPXV in patient lesion samples. Results are expected to provide real-world evidence of the suitability of lab-developed assays for detection of OPXVs and understand logistic challenges that will be faced. These updates will be presented at the 2020 ACVVR meeting.
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 prototype of a stand-alone kit for orthopoxvirus detection has 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 40 minutes using rabbit polyclonal antibodies against vaccinia virus as a capture reagent immobilized on a carrier, on the one side, and as a detection reagent associated with colloidal gold, on the other side. Evaluation of the effectiveness of detection of vaccinia virus (strain LIVP), cowpox virus (strain GRI-90), and ectromelia (strain K-1) has demonstrated the sensitivity of virus detection in unpurified cell culture preparations to be 103-104 PFU/ ml. The test does not detect any cross-reactions with heterogeneous viruses (measles, rubella, and varicella) that cause exanthematous diseases. The completeness of the assay, ease of analysis, and the ability to visually record the results make it possible for the test to be used in an off-laboratory setting.

The kit makes it possible to successfully detect viruses in clinical material, including blood, skin rashes, and organs, from infected animals (rabbits and mice), as well as to detect VACV in the crusts from pustules at the vaccination site in patients.

The plan of further work includes optimization of the kit’s manufacturing technology and design of its accessories; manufacture of experimental batches of the kit and their testing using a wide range of orthopoxviruses (including variola and monkeypox viruses highly pathogenic for humans), as well as the development of a codes and specifications package for the kit’s manufacture.