WHO Advisory Committee on Variola Virus Research

Report of the Twentieth Meeting

Geneva, Switzerland

26 – 27 September 2018
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EXECUTIVE SUMMARY

The Advisory Committee on Variola Virus Research held its twentieth meeting on 26 and 27 September 2018 at WHO headquarters in Geneva. The Committee acknowledged its role in preparing for the discussion on smallpox at the Seventy-Second World Health Assembly in May 2019 by reviewing progress on the live variola virus research agenda.

Achievements and considerations for variola virus research

The Committee unanimously recognized the extraordinary achievements of the research using live variola virus it had authorized, and considered that it continues to meet its commitments to ensure the delivery of medical countermeasures against smallpox.

As a result of the scientific work done under its auspices, Member States now have a range of public health tools to respond to a re-emergence of smallpox, which may also benefit the diagnosis, prevention and treatment of other orthopoxvirus infections. Most attending members of the Committee considered that live variola virus is needed for further development of antiviral agents.

Variola virus repositories

The Committee received reports on the status of the variola virus collections held at the two WHO Collaborating Centres that are authorized as repositories of variola virus, the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America, and the State Research Centre of Virology and Biotechnology (VECTOR), Koltsovo, Novosibirsk Oblast, Russian Federation. The number of stocks remained unchanged from last year in both repositories.

The reports of the respective repository inspections in the 2016-2017 cycle have been published. For the next cycle, inspections are planned for VECTOR in January 2019 and for CDC in May 2019.

Global health security

The Committee was informed that the WHO Smallpox Vaccine Emergency Stockpile remained unchanged in size and composition from the previous year and that the Secretariat was planning simulation exercises to test the procedures for the emergency use of smallpox vaccines.

The Committee acknowledged the establishment of the WHO Strategic and Technical Advisory Group for Infectious Hazards, whose remit included diseases that could threaten global health security, such as smallpox. The Committee appreciated receiving information on a monkeypox outbreak in Nigeria, as smallpox medical countermeasures could be relevant for monkeypox control.

Research update

The Committee received detailed updates on the progress of previously approved research using live variola virus. Ten research proposals had been received in 2018 from the two WHO Collaborating Centres authorized to hold variola virus. The Committee suggested that the work for one of the projects be undertaken collaboratively by the two Collaborating Centres.

Diagnostics

Researchers at VECTOR reported on development and use of a new multiplex real-time PCR technique and reagent kit for species-specific identification of human pathogenic orthopoxviruses and described investigation of variola virus strains from geographical regions not previously studied.
CDC researchers reported the development of multiplexed assays specific for variola virus for application in automated diagnostic platforms for use in remote areas and protein-based tests for variola virus. CDC was collaborating with partners in the Democratic Republic of the Congo on the field application of a commercial assay to detect monkeypoxvirus.

**Antiviral agents**

The Committee received updates on research on antiviral agents. An oral formulation of tecovirimat was approved for treatment of smallpox by the US Food and Drug Administration (FDA) in July 2018. The manufacturer was also developing an intravenous formulation.

Researchers at VECTOR continue to develop NIOCH-14, a compound that is structurally similar to tecovirimat, as well as a range chemical compounds and monoclonal antibodies.

Researchers at CDC are investigating monoclonal antibodies and mixtures thereof to neutralize variola virus in vitro and undertaking post-exposure prophylaxis efficacy studies in animals. CDC researchers are assessing the usefulness of humanized mice for evaluating anti-variola virus agents.

The manufacturer of brincidofovir reported progress on development of this agent that acts against variola virus by a different mechanism to that of tecovirimat. It is available in liquid and tablet form, with an intravenous formulation in development. The activity profile of this antiviral includes inhibition of orthopoxvirus replication.

**Vaccines**

With regard to vaccines, researchers at VECTOR had engineered a strain of vaccinia virus that was more immunogenic than the parent strain and led to reduced reactogenicity.

Researchers at both CDC and VECTOR have been investigating the neutralizing activity of sera of vaccinated subjects and animals, with CDC optimizing an assay to support vaccine studies.

A non-inferiority trial of two smallpox vaccines suggested that the MVA vaccine may be used in some subjects for whom ACAM2000 may pose some residual risk.

Following a transfer of business in Japan, the new manufacturer of the third-generation smallpox vaccine LC16m8 assured the Committee that it would continue producing vaccine for national and WHO stockpiles.

**Approval of smallpox countermeasures**

An overview was given of FDA's role in the development of smallpox medical countermeasures for approval, licensure or clearance, highlighting recent landmarks including approval of a real-time PCR assay for variola virus and of tecovirimat for smallpox.

**Conclusion**

The Committee recalled its prior recommendations on future research using live variola virus and considered the status of ongoing research. For treatment of smallpox, most members of the Committee who were present at this meeting considered that live variola virus is still needed for development of an additional antiviral agent with a mode of action that differs from that of the new antiviral compound approved in July 2018. With regard to diagnostic assays essential for public health, the Committee was divided on the question of whether use of live variola virus remained necessary. For further development of smallpox vaccines, a large majority of the members of the Committee considered that live variola virus was no longer needed.
MEETING PROCEEDINGS

Secretariat reports

1. Opening of the meeting and report of the Secretariat

The WHO Advisory Committee on Variola Virus Research met on 26 and 27 September 2018 under the chairmanship of Professor Geoffrey Smith with Mr David FitzSimons as Rapporteur. The Committee adopted its agenda (see Annex 1).

Dr Sylvie Briand (Director, Department of Infectious Hazards Management, WHO Health Emergencies Programme) opened the meeting. She recalled that the Committee had met annually since 1999. The crucial research that it had approved had been very successful: there were currently several licensed vaccines; high-quality diagnostics approved for orthopoxviruses generally and variola virus specifically; and one antiviral agent against variola virus (tecovirimat) had recently been approved and another was progressing towards licensure. Much credit and congratulations were due to all involved in these major advances.

Despite the progress, public health challenges remain. The threat of re-emergence of smallpox due to the accidental release of variola virus, although unlikely, still existed. A more recent concern was the growing potential for a deliberate attempt to recreate or manipulate variola virus by synthetic biology. Other public health threats included outbreaks of monkeypox in Africa, in particular the current outbreak in Nigeria, where none had been reported since the 1970s. Cases linked to this outbreak had been imported into the United Kingdom of Great Britain and Northern Ireland. All these concerns have spurred the WHO Health Emergencies Programme to intensify its work on preparedness and outbreak response, at both local and global levels.

Dr Briand emphasized the importance of the Committee’s role in preparing for the Seventy-Second World Health Assembly in May 2019 by reviewing progress towards the completion of the live variola virus research agenda. Member States would discuss the provisional agenda item on the timing of the destruction of variola virus stocks. The Committee’s report and recommendations would provide essential input into their discussions.

She welcomed the two new members of the Committee, Dr Inger Damon (Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America) and Dr Tan Wenjie (Chinese Center for Disease Control and Prevention, Beijing, China).

Within WHO, Dr Asheena Khalakdina, the Technical Officer in charge of the WHO Smallpox Secretariat for the past three years, was taking up another position in the Organization. Dr Briand thanked her for her work and introduced her replacement, Dr Rosamund Lewis.

Dr Khalakdina reviewed the planning for the discussion of the substantive agenda item on the timing of the destruction of variola virus stocks at the Health Assembly in May 2019. The Health Assembly’s resolution WHA49.10 in 1996 had allowed temporary retention for essential public health research. Since then, the Committee had regularly reviewed, approved and monitored research with live variola virus. The Committee at its previous meeting had proposed that all its members rather than a subcommittee would review proposals for research from the two WHO
Collaborating Centres.¹ At the current meeting, members of the Committee would review the proposals approved in 2018, for which research is underway, and one new proposal (see below).

The Committee had also overseen the biennial biosafety inspections of the two authorized repositories of variola virus stocks. The reports of the latest round of inspections in 2016-2017 were available on the WHO website.² The next round was planned for the first half of 2019.

The Committee would be updated on the status of the WHO stockpiles of medical countermeasures, including the physical inspection of the secure storage facility in Switzerland (see below). The Committee would also consider monkeypox in Africa in light of the recent outbreaks and the relevance of the research agenda on variola virus to this and other orthopoxvirus infections. WHO was working closely with CDC on developing a manual and toolkit for training purposes.

As mandated, the Committee had submitted progress reports on the status of research annually to the World Health Assembly. The Committee now had to make a clear statement about whether live variola virus was needed for essential public health research on medical countermeasures against smallpox and make a firm recommendation to the Health Assembly in 2019.

The Committee meeting report was aimed at decision-makers and general readership at global level.

**Monkeypox**

Through a video conference, Dr Olusola Aruna (on behalf of Dr Chikwe Ihekweazu, Director General, Nigeria Centre for Disease Control) gave an update on the ongoing outbreak of monkeypox in Nigeria and the public health response. The outbreak, caused by the West African clade of monkeypox virus as in the last outbreak in 1978, started in September 2017 and had peaked, with sporadic cases still being notified regularly. Some clusters were located in prisons, suggesting human-to-human transmission. At the time of the meeting, there had been 115 confirmed cases among 272 suspected cases, with seven deaths (four in HIV-positive subjects). Most cases have been in people aged 21-30 years. Two cases with travel from Nigeria were reported in the United Kingdom of Great Britain and Northern Ireland (UK), with subsequent infection of a health care worker.³

Public health responses, with support from the CDC (for example of viral sequencing), have included systems for managing samples, building diagnostic capacity, strengthening surveillance, training workshops and interim guidelines, learning from the CDC experience with use of smallpox vaccine in monkeypox outbreaks in the Democratic Republic of the Congo, cross-border collaboration, and contact tracing (family members and flight contacts for the two subjects who travelled to the UK). In Nigeria, the ministries of agriculture and the environment are cooperating with the health ministry on surveillance and control. Identified needs include a seroprevalence survey of the general population, better understanding of the natural history of the disease, further strengthening and maintenance of surveillance capacity, and studies of the animal reservoir and viral ecology.

Members of the Committee raised several questions and concerns. These included the fact that most monkeypox virus infections were in people born after the eradication of smallpox, what

³ At the time of the presentation, another case had just been reported in Israel related to travel from Nigeria.
contribution HIV infection might have, and the need to include monkeypox in the list of notifiable diseases in Nigeria. The Committee appreciated the relevance of the information on monkeypox virus, as medical countermeasures developed to detect, treat or prevent variola virus infections and disease could be relevant for monkeypox virus.

**Strategic and Technical Advisory Group for Infectious Hazards**

Dr Nahoko Shindo (Department of Infectious Hazards Management, WHO) described the scope, purpose and work of the newly established Strategic and Technical Advisory Group for Infectious Hazards. It provides independent advice and analysis to WHO on more than 20 pathogens that could threaten global health security, including variola virus and monkeypox virus. It intends to adopt the mechanisms of the Strategic Advisory Group of Experts on immunization, with working groups, and to have more frequent engagement of members between meetings.

2. Update on research proposals submitted to WHO for 2018 - Professor Geoffrey Smith

The Chairman recalled that the Committee had proposed at its nineteenth meeting in 2017 that the whole membership would review proposals submitted from the two repositories for research with live variola virus. By early 2018, five proposals had been received from CDC and four from VECTOR (see Annex 2); through written consultation, all had been recommended for approval by responding Committee members, and approval had been provided in writing to each Collaborating Centre. With regard to a CDC proposal for use of humanized mice as an animal model, although some reservations had been expressed about its validity and usefulness, a majority of respondents recommended approval.

Following a request from the United States Food and Drug Administration (FDA) after its approval of tecovirimat, CDC submitted a further proposal to obtain supportive information about heterogeneity of the gene target and the potential for drug resistance. Respondents agreed that the work should be done and recommended approval of research with six strains of live variola virus with known sequences available in the WHO Collaborating Centre freezer stocks at CDC. During discussion at the current meeting, the Committee was reluctant to endorse alterations of another orthopoxvirus to include the tecovirimat target sequence of interest for the two haplotypes that are not within the CDC stocks, as current WHO recommendations do not allow such manipulations.

As one haplotype is available within the freezer stocks at VECTOR, the Committee recommended that the two WHO centres - CDC and VECTOR - undertake the work collaboratively, sharing protocols. FDA should indicate whether the results were satisfactory; if not, further work could be proposed and discussed subsequently.

By consensus, the Committee endorsed continuation of nine projects, for which progress was reviewed at this meeting, and recommended partial approval of the tenth proposal subsequently submitted, as outlined above.

All members of the Committee were encouraged to respond to the request to review proposals.

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1. Letters of approval of research for 2018 from the WHO Smallpox Secretariat were sent to CDC in February 2018 and VECTOR in April 2018.
3. Update on Smallpox Vaccine Emergency Stockpile – Mr Tim Nguyen

Mr Nguyen updated the Committee on the status of the emergency stockpile. The WHO stockpile exists in two forms: a physical stockpile held in Switzerland, and pledged stocks held by donor countries. The physical stockpile consists of about 2.8 million doses of first- and second-generation vaccines. In July 2018 the content was inventoried. The pledged stockpile comprises first, second- and third-generation vaccines, with altogether about 27 million doses, available on request by WHO. Vaccines in both stockpiles are regularly tested for potency. The Secretariat was planning simulation exercises to test the procedures for the emergency use of smallpox vaccines.

In discussion, the issue of new vaccines differing from the standards originally applied for first-generation vaccines was raised. The Secretariat planned to look into the matter and would report back to the Committee. The Committee and the Secretariat concurred that the total stockpile represented a limited supply in case of an emergency, raising issues of governance, decision-making and equity of access as well as approaches to replenishing the stockpile.

Further issues raised concerned the prevalence of HIV infection in some countries, possibly complicating the use of smallpox vaccine. The Committee concluded that guidance was needed.

4. Update on the variola virus repositories biosafety inspection visits – Dr Kazunobu Kojima

Dr Kojima (WHO Health Emergencies Programme) reviewed the 2016-2017 cycle of the biosafety inspection visits to the variola virus repositories at VECTOR and CDC. The inspection team noted that most findings from the previous inspection had been addressed successfully. During the most recent inspections, the team identified no priority 3 findings and only a few priority 2 findings (i.e. needing timely corrective action).

The reports of the two inspections were published in 2018 after scientific redaction. Since then, action plans have been received from both repositories, showing a high level of commitment. For the next cycle, inspections are planned for VECTOR in January 2019 and for CDC in May 2019.

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5. Report on the variola virus collection at the WHO Collaborating Centre Repository in Vector, Koltsovo, Novosibirsk Region, Russian Federation – Dr Rinat Maksyutov

Dr Maksyutov confirmed that the work on the approved research projects with live variola virus complied with national and international requirements and that the conditions of storage of the virus were rigorous and secure. The collection comprises 120 strains from countries on four continents, with the number of stocks remaining unchanged over the past year.

The approved research with live virus focused on: novel chemical antiviral agents against variola virus; replenishment of the stocks of non-infectious material derived from live variola virus for diagnostics development; evaluation of antiviral monoclonal antibodies; and the development of less-reactogenic fourth-generation vaccines.

Preclinical trials of one compound, NIOCH-14, were being finalized, and a further 66 new chemical entities and two human monoclonal antibodies were being tested against orthopoxviruses. Five strains of variola virus are being grown for DNA preparation and sequencing. The neutralizing activity of a highly immunogenic, attenuated vaccinia virus vaccine (VACdelta6) was being studied in animal models (see section 12 below).

6. Report on the variola virus collection at the WHO Collaborating Centre for Smallpox and Other Orthopoxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America - Dr Vicki Olson

Between 2017 and 2018 no new variola virus seed pools were added to the collection and there were no withdrawals. WHO was informed of a closely monitored, successful transfer of stocks following a freezer malfunction. A complete inventory of laboratory working stocks was made in August 2018. Approval for five research proposals was received in early 2018. Work commenced in February, paused for annual maintenance of the containment facility, and resumed in June 2018.

Having optimized protocols for sequencing DNA from variola virus, 42 sequences have been determined since October 2017. Phylogenetically, the newly isolated sequences continue to group geographically within known clades. About 30 more viral sequences remain to be analysed.

After the approval of tecovirimat, FDA requested its further evaluation against variola virus strains with all known haplotypes of the target gene, to ensure that none confer resistance to the drug.

7. Use of live variola virus to maintain and regenerate non-infectious variola-derived materials for diagnostic development support – Dr Vicki Olson

Dr Olson also presented an update on nucleic acid-based diagnostics. In the USA, FDA has approved several diagnostic assays specific for orthopoxviruses, including variola virus, for use in the Laboratory Response Network. These assays have been transferred to a multiplexed format with a view to adapting to an automated diagnostic platform for use under field conditions where power is available. The multiplexed assays specific for variola virus retained the sensitivity seen when conducted individually. The automated cartridge-based nucleic acid amplification test (NAAT) diagnostic platform to which the assays are being adapted offers several advantages, including compactness, thermostable reagents and minimal sample preparation. The technology has been successfully applied in the Democratic Republic of the Congo for detection of monkeypox and other disease outbreaks, expanding the potential for surveillance. CDC was working with commercial
companies to optimize the delivery in pre-packaged cartridges of diagnostic reagents for detection of variola virus and other orthopoxviruses.

In discussion, Dr Olson confirmed that the PCR assays recognize all strains of variola virus sequenced so far. Recognizing the challenges of field use, such as minimal availability of biosafety training and equipment (containment and personal protective equipment), she emphasized that work was being done on reducing the number of sample manipulations to a minimum to improve safety. Challenges also exist to ensure reliable sources of power, such as the need for an electricity generator.

8. Replenishment of the stocks with non-infectious material, derived from variola virus, required for diagnostics development – Dr Sergei Shchelkunov

Dr Shchelkunov reviewed investigations at VECTOR into the genomes of variola viruses and their various phylogenies. A new multiplex real-time PCR technique and reagent kit, Vector-MPCR, Smallpox, have been developed for species-specific identification of human pathogenic orthopoxviruses. Work was underway with five strains of variola virus held in the repository at VECTOR from geographical regions not previously studied, for full-length genome sequencing and testing of the extracted DNA samples with the help of the reagent kit.

9. Development of protein-based diagnostic and detection assay specific for variola virus – Dr Vicki Olson

Dr Olson reviewed the different benefits and limitations of protein-based and nucleic acid-based tests for variola virus. Protein-based tests overcome many challenges that exist for diagnostic use in resource poor settings and have good potential for use without laboratory equipment or infrastructure, but require species-specific monoclonal antibodies. CDC has been producing variola virus-specific antibodies and obtaining reproducible results in detecting live virus with lateral flow assays. Work continued to investigate combinations of monoclonal antibodies with maximal sensitivity and specificity.

In collaborative work, CDC was evaluating different detection methods and point-of-care formats, including immunofiltration with orthopoxvirus-generic monoclonal antibodies, and the feasibility of their use. To understand the challenges better, CDC was working with partners in the Democratic Republic of the Congo on the logistics and field use of a commercial lateral flow assay to detect monkeypox virus in patients’ samples. The work should provide insights into designing strategies for deploying diagnostics into remote regions, thereby enhancing preparedness and response activities.

CDC was also studying the use of a variola virus-encoded protein microarray for evaluation of antibody responses. It had used such an assay to evaluate suspect monkeypox cases and to differentiate the response to vaccination with vaccinia virus from infection with other orthopoxviruses.

These approaches to building rapid and accurate diagnostic capacity across the world were crucial to contributing to successful disease containment in the event of a re-emergence of smallpox and effective orthopoxvirus disease control.

In discussion, members of the Committee asked about relative costs of different assays, the volume of demand, cross-reactivity, the possibility of using capture methods with polyclonal rather than monoclonal antibodies, the challenges of electricity and supply of reagents, and maintenance. Protein-based diagnostic assays obviate many of the challenges which exist for diagnostic assay detection within remote areas; requiring no electricity, little to no infrastructure, and minimal technical training. One such diagnostic assay is the lateral flow assay, which is rapid but requires
improvements, especially in sensitivity. CDC has focused on improving sensitivity for variola virus over other orthopoxviruses.

**Antiviral agents**

10. Discovery and testing of novel chemical antivirals and antivirals based on monoclonal antibodies for smallpox treatment and prevention – Dr Elena Gavrilova

Dr Gavrilova informed the Committee that a finished dosage form of the compound NIOCH-14 for oral administration had been formulated as capsules. The antiviral agent was being manufactured according to the requirements of Good Manufacturing Practices in order to finalize preclinical studies and conduct clinical trials. Some 66 new chemically synthesized compounds of different classes had been tested against vaccinia, cowpox and ectromelia viruses in vitro, and investigation of the antiviral activity of the most promising compounds against variola virus was ongoing. Monoclonal antibodies targeting orthopoxviral proteins encoded by two open reading frames were being tested for neutralizing activity in surrogate orthopoxvirus models.

Members of the Committee noted that NIOCH-14 was structurally similar to tecovirimat and might have the same mechanism of action; the potential for development of resistance would also be expected to be similar. It was suggested that these questions could best be answered by VECTOR working with tecovirimat; sharing protocols and materials would be a good collaborative exercise.

Questions were also raised about an apparent inverse relation between dose and bioavailability. With regard to further preclinical studies, Dr Gavrilova said that it was planned to progress from ectromelia virus to monkeypox virus. Development to licensure could take some time, it was noted.

11. Use of live variola virus to evaluate antiviral agents (monoclonal antibodies) against smallpox – Ms Ashley Kondas

Ms Kondas outlined the arguments in favour of developing more than one antiviral agent against variola virus: it may be possible for variola virus to develop resistance; advances in synthetic biology reinforced the need for multi-therapeutic approaches; and vaccinia immune globulin was in limited supply. Monoclonal antibodies against orthopoxviruses neutralize many such viruses. She presented evidence that such human monoclonal antibodies from three different entities, whether alone or in cocktails, prevent in vitro multiplication of variola virus.

She also presented data on the use of a mixture of monoclonal antibodies, Mix 4, in the monkeypox virus (MPXV)/prairie dog model, showing that the monoclonal antibody mixture reduced morbidity and increased survival when given in two doses: one prophylactic and one post-exposure.

Experience from other diseases with outbreak potential showed that, despite the progress represented by the FDA approval of tecovirimat, a multi-therapeutic approach gave significant benefit. Further evaluations of monoclonal antibody mixtures were needed to validate their value as a therapeutic tool against smallpox.

In response to questions, Ms Kondas agreed that it would be easy to determine the half-life of the monoclonal antibodies used in Mix 4 during the MPXV challenge study, which were still detectable seven days after administration. The antibody mixtures had been designed to be generic against orthopoxviruses, but the potential remains that one specific for variola virus could be designed.
Vaccines

12. Assessment of the neutralizing activity of sera from vaccinated animals using variola virus to support the development of less-reactogenic fourth-generation vaccines – Dr Sergei Shchelkunov

Dr Shchelkunov described work being done to reduce the reactogenicity of smallpox vaccines by deleting genes from the parent vaccinia strain used in the former Soviet Union for vaccination against smallpox.

Previous research had developed a vaccinia strain with deletion of five virulence genes and recently another strain, VACdelta6, had been created with the deletion of a further gene, A35R, which controls the presentation of antigens by the major histocompatibility complex class II. The resulting new strain can replicate in mammalian cells, was more immunogenic than the parent strain, led to reduced reactogenicity and was more protective than previous viruses. High levels of protection were seen in all three animal models tested (rabbits, mice and guinea pigs), and the sequence of the new strain has been recorded in GeneBank.

As a conclusion to preclinical studies, the neutralizing activity of sera of animals vaccinated with VACdelta6 would continue to be studied in the second half of 2018. Clinical trials are envisaged for 2019 onwards.

13. Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of third-generation vaccines – Dr Christina Hutson

The plaque reduction neutralization test (PRNT), which measures the ability of immune sera to neutralize mature variola virus forms in vitro, has been used as the endpoint in the evaluation of vaccines, in particular for third-generation smallpox vaccines. Because different viral strains had been found to give significantly different results in the assay (indicating that a related orthopoxvirus could not be used as a surrogate to show efficacy against variola virus), researchers at CDC optimized the PRNT for intracellular mature variola virus. Dr Hutson described recent developments.

In 2018, FDA approved two submissions from CDC in collaboration with the manufacturer: a redevelopment report for improving the methods used in the PRNT and an analytical plan for testing samples of sera from clinical trials with the variola virus PRNT.

Dr Hutson reported that work has been completed on testing samples from a non-inferiority trial comparing the MVA and ACAM2000 vaccines, and that the data were being analysed. In addition, work was undertaken to refine the PRNT for the extracellular enveloped variola virus so as to be more similar to the 12-well assay for intracellular mature variola virus. The optimized test was applied to a small set of sera from subjects vaccinated with LC16m8 or Dryvax vaccine. Preliminary data showed that sera from Dryvax subjects neutralized extracellular enveloped variola virus more efficiently than those from LC16m8 vaccinees. The findings need to be substantiated as the neutralizing ability of sera from different vaccine regimens needed to be known for future submissions for regulatory approval of third-generation vaccines.

Animal models, product development and licensure

14. Use of live variola virus to determine whether mice are a suitable animal model for human smallpox – Dr Christina Hutson

As no satisfactory animal model of smallpox exists for evaluating antiviral agents, researchers at the WHO Collaborating Centre in CDC have looked at humanized mice. Dr Hutson reviewed work with three different types of humanized mice. Humanized mice intranasally challenged with variola virus
showed clinical signs requiring euthanasia, with an incubation period of 13-21 days, making them a good model for testing therapeutic agents. Research had shown that a human component in these mice was necessary for the extensive spread of variola virus to most tissues tested that was seen after challenge with live virus, including tissues of the nose, bone marrow, liver and spleen. Subsequently, studies to further characterize the variola virus/humanized mouse model were undertaken so that the FDA may assess its usefulness to evaluate antiviral candidates and other medical countermeasures.

In discussion, it was observed that the model should also generate a better understanding of what immune responses follow infection with variola virus and what favours viral pathogenesis in humans.

15. Update on MVA smallpox vaccine – Dr Heinz Weidenthaler
Dr Weidenthaler reported that the manufacturer of the highly attenuated MVA vaccine, had recently completed a non-inferiority study comparing two doses of that vaccine with one dose of ACAM2000. The study had two primary endpoints: an immunological correlate of protection (peak serum titres of neutralizing antibodies) and a surrogate of efficacy (demonstration that vaccination with MVA before administration of ACAM2000 resulted in an attenuation of “take” in terms of maximum lesion area). Both endpoints were met. MVA vaccine was safe and well-tolerated, with better tolerability than ACAM2000, although ACAM2000 was significantly better tolerated following MVA priming compared to ACAM2000 as a single vaccination. Findings of a phase II trial that included HIV-positive subjects suggested that MVA vaccine may be used in the general population, including in persons for whom vaccination with ACAM2000 may pose significant risks.

The manufacturer was currently preparing a Biologics License Application for a liquid-frozen formulation for submission to FDA later in 2018. The company has delivered 28 million doses (in liquid form) to the United States National Smallpox Stockpile and, for the long term, has contracted with the US Government to provide 132 million doses in a freeze-dried formulation to protect 66 million citizens with MVA vaccine.

In discussion, it was observed that data from the non-inferiority trial were the final requirements before licensure. Members of the Committee recognized the positive and valuable communication between company and the regulatory authorities in both Europe and the USA. With regard to duration of immunity after vaccination, the kinetics of the immune response after a booster dose indicated immunity was retained.

16. Progress towards regulatory approval of tecovirimat (ST-246) – Dr Dennis Hruby
Dr Hruby recalled the characteristics of tecovirimat and the product development path that led to approval of an oral formulation for the treatment of smallpox by FDA in July 2018. That represented the first-ever approval of a small molecule for use in humans on the basis of the FDA Animal Rule, following the establishment and maintenance of a successful public-private working team. The manufacturer had pledged to undertake several post-marketing studies, including pharmacokinetics, drug-drug interactions, characterization of activity against an expanded panel of variola virus isolates and recombinant vaccinia viruses, and environmental impact.

The company was developing an intravenous formulation for individuals unable to benefit from the oral form and for inclusion in the US National Smallpox Stockpile. A single ascending dose clinical trial showed a linear response. No serious adverse effects were seen. The company was working on

1 MVA-BN® approved as IMVANEX® in Canada and IMVAMUNE® in the European Union.
commercial-scale development for an eventual New Drug Application to FDA. It was also exploring the possible use of tecovirimat against other orthopoxvirus infections, including monkeypox.

Comments in discussion included a request for further information about the environmental impact assessment. That was a relatively new FDA requirement and was still being considered. With regard to administration of the antiviral to young children, the company was developing a protocol for opening packages for mixing with foods or milk in order to obtain the appropriate dose. Alternatively, the liquid formulation existed and was stable for at least five years. Pricing was still under discussion within the company. Issues of licensing for use in different countries would need to be resolved and countries would need to be made aware of what resources and medical countermeasures were now available.

17. Update on the development of brincidofovir for smallpox – Dr Scott Foster
Dr Foster outlined progress in the development of brincidofovir whose activity includes inhibition of orthopoxvirus replication. Its target product profile included treatment of smallpox and adenovirus infection in high-risk paediatric transplant recipients. It had acceptable safety and tolerability and was available in liquid formulation and as tablets (including blister packaging). An intravenous formulation was in clinical development. The company was completing its manufacturing readiness processes.

Brincidofovir has a different mechanism of action from that of tecovirimat and combination of the two antiviral agents has been shown to be effective both in vitro and in a mouse model.

Progress was being made towards approval for use against smallpox, including cooperation with the Biomedical Advanced Research and Development Authority in the USA. FDA had agreed that the rabbitpoxvirus/rabbit and ectromelia virus/mouse models were suitable for generating data for submission under the Animal Rule. Brincidofovir had been designated an orphan drug (by the European Medicines Agency in 2016 and by FDA in June 2018). The company was in discussions about placing the antiviral in national and regional stockpiles.

Comments in discussion related to the feasibility of administering the antiviral with a vaccine without compromising the response, dosage, and the unlikely prospect of resistance.

18. Update on the third-generation smallpox vaccine LC16m8 – Dr Yashihiro Shinmura
Dr Shinmura informed the Committee of the transfer of the main business activities of the manufacturer in Japan, including the production of the smallpox vaccine LC16m8, to another company in July 2018. There would be no change in the facilities, capacity and quality of LC16m8 vaccine. The new manufacturer would continue the previous contribution to national security and national defence by manufacturing vaccines for smallpox stockpiling. WHO and the Japanese Government had resumed discussions about potential donations to the WHO stockpile.

In Japan, a Smallpox Vaccine Research Group had been established, with representatives from government, industry and academia; its main objective was to obtain data on stability of LC16m8. The approved shelf life of the lyophilized vaccine was four years when stored at -20 °C (and two years at 5 °C). However, data show that when stored at -20 °C, vaccine potency is retained at 10 years. Even at -80 °C for five years, the vaccine showed no loss of potency. The bulk vaccine product had also been authorized for storage for five years at -80 °C, meaning that the production lead time can be shortened to several months thereby allowing greater supply of the drug product in a shorter period of time.
Members of the Committee noted that delivery of the vaccine by bifurcated needles allowed a reduction of dose and that it was easier to train health care workers in that use. Concern was expressed about the fact that there were only two smallpox vaccine producers, and members asked how long the new manufacturer would continue to make LC16m8 vaccine and what were the plans for maintaining manufacturing capacity (million doses a year). Dr Shinmura assured the Committee that no changes to previous practice were envisaged and that every year the health ministry decided on vaccine production and reviewed quality reports. Both vaccine stored for 10 years and vaccine produced from bulk were subjected to quality control.

19. FDA perspective on the development and approval of smallpox medical countermeasures – Dr Edward Cox

Dr Cox outlined FDA’s role in the development of smallpox medical countermeasures in order that they qualify for and receive FDA approval, licensure or clearance. In close collaboration with the developers of smallpox countermeasures FDA had established feasible and appropriate regulatory pathways, providing feedback on proposed studies.

He recalled the remarkable list of antiviral agents, vaccines and diagnostic tests that had been developed. Markers of recent progress included the granting in 2017 of a de novo petition to CDC for a variola virus real-time PCR assay and approval in 2018 of tecovirimat, the only antiviral agent with an indication for smallpox. He highlighted the use of surrogate viruses in developing antiviral agents. FDA had granted a Pre-Emergency Use Authorization for the MVA vaccine. He advised that licensure of next-generation smallpox vaccines should be based on the traditional approval pathway, when possible, such as for example non-inferiority trials.

The Animal Rule was an evolving approach to assessing efficacy; results from animal studies were supportive but did not rule out the need for human data. Clinical trials and post-marketing studies provided excellent information. Given the experience of combination therapy in other diseases, the US FDA would need good suitable data for smallpox treatment, even if not from human subjects. In July 2018 FDA issued draft guidance for industry for developing drugs for treatment and prevention of variola virus infection.

Members of the Committee underlined the scale of the research achievements for smallpox. For antiviral agents, they concurred that, even if only one were available, clinical trials were necessary to assess safety. For further drugs or combination therapy, different and simple designs of trials should be prepared in advance. Determination of the human dose from animal studies would need further research into modes of action. No correlates of protection had been studied or identified during the eradication of smallpox. Members of the Committee noted that the European Commission had set guidelines and a requirement to conduct small trials with a single arm, and that was being done in the Democratic Republic of the Congo for use of MVA vaccine against monkeypox. They also raised the question of how smallpox medical countermeasures would be evaluated.

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GENERAL DISCUSSION

The decisions of the Forty-ninth World Health Assembly in 1996 (resolution WHA49.10) and the Fifty-second World Health Assembly in 1999 (resolution WHA52.10) to temporarily retain stocks of variola virus for essential work with live variola virus for public health benefit were based on the state of scientific knowledge and technologies at the time.

The Committee’s role was to contribute technical input to the discussion on the timing of destruction of live stocks of variola virus by Member States at the Seventy-second World Health Assembly in May 2019. It aimed to provide the best guidance on safety and biosecurity, risks, global public health, the status of essential research and what more research was needed, especially in light of developments such as synthetic biology and the recreation of viruses including an orthopoxvirus. It was recognized that there were divergent and valid scientific arguments about research needs and interpretation of results.

In September 2013 the majority of the members of the Advisory Committee at its fifteenth meeting considered that live variola virus was needed only for the further development of antiviral agents against smallpox. Conversely, in November 2013, the Advisory Group of Independent Experts which was convened to review the smallpox research programme opined with a majority view that there was no need to retain live variola virus for further development of antiviral agents against smallpox. Neither conclusion was reached unanimously among respective committee members.

As technology has progressed, research using live variola virus has been approved by the Advisory Committee, primarily to conclude already-approved projects, and focus on development of antiviral agents. The Committee recognized that, given the decision of the Health Assembly to destroy live virus stocks, the situation could continue for only a limited period and that research must be prioritized with defined timelines.

The Committee was cognisant of the benefits of having eradicated a devastating and often fatal illness and the arguments behind the World Health Assembly decision for destroying its causative pathogen. Destroying the live virus stocks should remove the risk of accidental release and guarantee the safety of the world’s population from a deadly disease. However, with the advent of synthetic biology,¹ the virus could now be recreated from known DNA sequences which are in the public domain. Some members were receptive to arguments for retention of live virus stocks, and by implication preserving research expertise and response capacity in the two WHO collaborating centres, including diagnostic capacity, the development of new antiviral agents, the ability to investigate any new synthetic virus, and the provision of well-defined reference standards for any subsequent research and development needs. It was noted that the absence of live virus or improved assays could deprive future generations of the possibility of applying technological advances for smallpox, which at times also benefit wider orthopoxvirus research. The question of whether we live with existing tools or continue to create products and devices with future state-of-the-art technologies was, however, not one for the Committee to answer.

More specifically, members of the Committee posed questions about whether more research was needed to improve existing tools or whether those tools would be sufficient to cope with any outbreak of variola virus infection today or in the future. Furthermore, was research, for example on

protein-based diagnostics, desirable or essential for public health? Several members of the Committee maintained that diagnosis of smallpox is first and foremost clinical; the rash is sufficiently distinctive. In addition, the WHO Secretariat as well as Member States had manuals and extensive collections of images to support clinicians in the field in making the diagnosis. The importance of initial clinical diagnosis, leading to subsequent isolation of the patient, reduced the importance of rapid diagnostic tests and confirmation from a public health perspective. On the other hand, public health authorities needed rapid and accurate alerts about any reappearance of smallpox or pox-like diseases. Clinicians needed certainty about what disease they were dealing with. Public health officials needed such knowledge to be able to communicate accurate information at all levels, including the risks for international travel and onward transmission.

One antiviral agent against variola virus (tecovirimat) was approved in 2018, but the potential for resistance inevitably exists. For that reason and following the example of antimicrobial treatment of diseases such as AIDS and malaria, Committee members argued for the development of another antiviral agent with a different mode of action from that of the available compound, and for consideration of combination therapy.

Advances in technology raised risks as well as opportunities for public health. The advent of, and access to, synthetic biology technologies has increased the risk of re-creation and/or manipulation of variola virus. Technological progress and easier access to equipment and reagents also heightened the risk of genetic engineering being used to create a variola virus with augmented virulence for the purposes of bioterrorism. The synthesis of an orthopoxvirus, reported to the Committee at its nineteenth meeting,1 and other viruses testified to the reality of this risk.

Diagnostics

The Committee discussed whether further work on developing a species-specific PCR test for variola virus was essential or merely desirable. It was recognized that in the field, supplies of electricity and reagents might not always be sustainable. Moreover, some equipment for PCR tests was no longer being produced and maintenance might not be guaranteed, consequently limiting the applicability of existing PCR-based diagnostics.

Several proposals were made. The authorized repositories in the Russian Federation and the USA hold respectively about 50 and 30 strains of variola virus that remain unsequenced; all these strains could and should be sequenced and viral DNA should be prepared from existing stocks. The work would take about one year. When completed, that would mean that all known existing stocks were sequenced. However, that raised the question of whether existing diagnostic tests would be able to detect all the newly determined sequences. On the other hand, knowledge of the sequences could further enable the recreation of variola viruses, which is prohibited by WHO guidelines.

Protein-based tests based on all known strains would eliminate the need for live virus, although advances in technology could, it was argued, necessitate the existence of live virus for further research. Variola viral DNA could be expressed. Another proposal was to modify the rules on genetic manipulation to allow variola genes that are unlikely to contribute to virulence to be expressed.

Vaccines
Most members of the Committee accepted that further work with live variola virus for the purpose of vaccine development was not necessary. Work to complete the current studies on LC16m8 was practically finished and would require about one year. The Russian Federation’s fourth-generation vaccine was completing preclinical studies. Researchers involved in vaccine development continue to explore development of existing vaccines to which further antigens can be added.

The Committee generally agreed that animal models of smallpox were inadequate for testing vaccines, and that the results of vaccine non-inferiority trials were accepted by regulatory authorities, such that variola virus infected animal models are not required for vaccine licensure.

Antiviral agents
Tecovirimat was approved by the FDA in July 2018. VECTOR’s NIOCH-14 is structurally similar and may have a similar mode of action. Brincidofovir was still on the path to licensure. The Committee was firmly of the view that having only one licensed antiviral agent was not sufficient, and it supported the goal of at least two such drugs with different modes of action. One class of such a new product was monoclonal antibodies – as described by CDC (see section 11 above). Concerns about development of resistance supported the exploration of combination therapy.

One proposal was for collaboration between the two repositories on investigating the mode of action of tecovirimat and NIOCH-14. Another proposal was to revisit the rules about inserting variola virus genes into another orthopoxvirus as an alternative to using live variola virus. There was no consensus on that approach, but it was strongly argued that testing compounds for anti-variola activity needed live virus not surrogate models.

Several antiviral candidates were in development. However, it was uncertain if any of these additional candidates would reach licensure and how long that might take. This uncertainty was common in the development of pharmaceuticals.

Committee members concluded that, although the humanized mice model is not useful for studying immune response, it has potential for evaluation of therapeutic agents against variola virus in vivo.

Achievements
The Committee reiterated the extent of the achievements. By 2018 one antiviral agent against smallpox had been approved (by FDA). A PCR-based nucleic acid diagnostic test had been approved (in both the Russian Federation and the USA), although it was not commercially available. Advances had been made towards a protein-based assay with potential for use as a point-of-care test. A third-generation vaccine, safer than the first-generation vaccines, has been licensed in several countries.\(^1\) Member States were now in a position to maximize public health benefit as a result of the scientific work done under the auspices of the Advisory Committee.

The members of the Committee were unanimous in recognizing the extraordinary achievements that had been made through authorized research, in particular with the culmination of projects in the past year and the potential impact of this science on public health questions.

These accomplishments represent several remarkable “firsts”: the development of an antiviral agent against a human disease that was eradicated and for which there was no adequate animal model; the continued improvement of vaccines against a disease with no patients; and completion of work

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\(^1\) Imvamune® in Canada and Imvanex® in the European Union. Work on a fourth-generation vaccine was underway in the Russian Federation.
using an agent that required containment in BSL-4 facilities. Members of the Committee applauded
the commitment and work of a small group of scientists, clinicians and other public health workers
and various governmental, academic and private sector entities.

The Committee considered that it had gone a long way towards meeting its commitments to
ensuring the delivery of medical countermeasures against smallpox. It recommended that the
translational benefit of the scientific work it had approved into the diagnosis and treatment of
monkeypox and other orthopoxviruses also be fully explored.

Further considerations
Other points raised in discussion included the need for further consideration of the following:

- the implications of the laboratory synthesis of a poxvirus, reported in 2016;
- further discussion of the appropriately stringent rules on manipulating variola virus and its
genes, and whether these rules could or should be modified so that non-pathogenic genes
could be expressed. Such work, to be conducted only in very strict conditions in the two
WHO collaborating centres and under the supervision of WHO, could lead to the
development of new technologies without requiring the use of live variola virus.
- The shelf-life of materials kept in global and national stockpiles. Members also raised the
issue of the fate of materials kept in stockpiles and their shelf-lives

These and other questions would benefit from further in-depth discussion in appropriate fora.

FUTURE RESEARCH USING LIVE VARIOLA VIRUS

In view of the forthcoming discussion at the Seventy-Second World Health Assembly in May 2019,
the Committee recalled its prior recommendations on future research using live variola virus.

In light of the findings reported in the meeting, the Committee underlined the real benefit and
practical utility of the work on variola virus, resulting in better emergency preparedness. The
Committee highlighted a recent example of an application in an outbreak of a re-emerging disease,
namely the use of diagnostic tools for early and rapid diagnosis as well as limited administration of
smallpox vaccine and antiviral agent in the recent and current outbreaks of monkeypox in Africa.

For treatment of smallpox, the majority of the members of the Committee considered that live
variola virus is still needed for further development of an additional antiviral agent with a mode of
action different to the recently licensed antiviral, tecovirimat.

For further development of diagnostic assays essential for public health, the Committee was divided
on the question of whether use of live variola virus remained necessary. Several members were
convinced that use of live virus was no longer needed. On the other hand, several other members
were of the view that it would be highly beneficial to develop simple point-of-care assays, in addition
to the approved laboratory PCR-based tests, in order to provide more rapid diagnosis in field
conditions. Development of some of these newer diagnostics might require use of live variola virus.

For further development of smallpox vaccines, a large majority of the members of the Committee
felt that live variola virus was no longer needed.

The Committee urged that the findings of the approved research using live variola virus be
disseminated to a broader audience including and beyond public health and technical experts, in
order that the actual and potential public health benefits arising therefrom may be more widely
understood and applied.
ANNEX 1. Agenda

Twentieth meeting of the WHO Advisory Committee on Variola Virus Research

26 to 27 September 2018

Agenda

DAY ONE – 26 September 2018

09:00 – 09:30 OPENING – Dr P. Salama, Deputy Director General, WHO Health Emergencies Programme

Dr M. Ryan, Assistant Director General, WHO Health Emergencies Programme

Welcome remarks – Dr S. Briand, Director, Department of Infectious Hazard Management, WHO Health Emergencies Programme

VARIOLA VIRUS REPORTS


09:45 – 10:15 Update on research proposals submitted to WHO in 2018 – G.L. Smith, Chair

10:15 – 10:25 Update on Smallpox Vaccine Emergency Stockpile – T. Nguyen

10:25 – 10:35 Update variola virus repositories biosafety inspection visits – K. Kojima

10:35 – 11:00 TEA/COFFEE BREAK

11:00 – 11:15 Report on the variola virus collection at the WHO Collaborating Center Repository in VECTORE, Koltsovo, Novosibirsk Region, Russian Federation – R. Maksyutov

11:15 – 11:30 Report on the variola virus collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA – V. Olson

VARIOLA VIRUS RESEARCH UPDATE – 2018

DIAGNOSTICS

11:30 – 11:45 Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support – V. Olson

11:45 – 12:00 Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development – S. Shchelkunov
12:00 – 12:15 Development of protein based diagnostic and detection assays specific for variola virus – V. Olson

12:15 – 12:30 Discussion on diagnostics

12:30 – 13:30 LUNCH

ANTIVIRALS

13:30- 13:45 Discovery and testing of novel chemical antivirals and antivirals based on monoclonal antibodies for smallpox treatment and prevention – E. Gavrilova

13:45 – 14:00 Use of live variola virus to evaluate antiviral agents (monoclonal antibodies) against smallpox – A. Kondas

14:00 – 14:15 Discussion on antivirals

VACCINES

14:15 – 14:30 Assessment of the neutralizing activity of vaccinated animals sera using variola virus to support the development of less reactogenic fourth-generation vaccines – S. Shchelkunov

14:30 – 15:00 TEA/COFFEE BREAK

15:00 – 15:15 Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of “third” generation vaccines – C. Hutson

15:15 – 15:30 Use of live variola virus to determine whether mice are a suitable animal model for human smallpox – C. Hutson

15:30 – 15:45 Discussion on vaccines

LICENSURE UPDATES

15:30 – 15:45 Update on MVA smallpox vaccine (MVA-BN® / IMVANEX® / IMVAMUNE®) – H. Weidenthaler

15:45 – 16:00 Progress towards approval and deployment of tecovirimat/ST-246 (TPOXX®, Arestvyr®) – D. Hruby

16:00 – 16:15 Update on the development of brincidofovir (CMX001) for smallpox – S. Foster

16:15 – 16:30 Update on the “third” generation smallpox vaccine LC16m8 – Y. Shinmura

16:30 – 16:45 FDA Perspective on the development and approval of smallpox medical countermeasures – E. Cox

16:45 – 17:30 General discussion

CLOSE OF DAY ONE
DAY TWO – 27 September 2018

Closed discussion for members and advisers of the Advisory Committee on Variola Virus Research

09:00 – 10:30  Monkeypox – current status in Nigeria and the West and Central African region

10:30 – 11:00  TEA/COFFEE BREAK

11:00 – 12:30  Discussion on status of smallpox preparedness and remaining variola virus research priorities

12:30 – 13:30  LUNCH

13:30 – 15:00  Discussion on recommendations of the Advisory Committee on Variola Virus Research for retention of variola virus stocks

15:00 – 15:30  TEA/COFFEE BREAK

15:30 – 17:00  Finalization of draft summary of the report of the Advisory Committee on Variola Virus Research and recommendations to the Director-General of WHO for the Seventy-second World Health Assembly

CLOSE OF THE TWENTIETH MEETING OF THE ADVISORY COMMITTEE ON VARIOLA VIRUS RESEARCH
## ANNEX 2. Research proposals for 2018

<table>
<thead>
<tr>
<th>Proponent and projects</th>
<th>Yes</th>
<th>No</th>
<th>Majority opinion and notes</th>
<th>Approval date</th>
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<tr>
<td><strong>VECTOR</strong></td>
<td></td>
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<tr>
<td>1. Discovery and testing of novel chemical antivirals for smallpox treatment and prevention</td>
<td>7</td>
<td>4</td>
<td>Yes</td>
<td>April 2018</td>
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<td>2. Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development</td>
<td>7</td>
<td>4</td>
<td>Yes</td>
<td>April 2018</td>
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<tr>
<td>3. Use of live variola virus to evaluate antivirals against smallpox based on monoclonal antibodies.</td>
<td>9</td>
<td>4</td>
<td>Yes</td>
<td>April 2018</td>
</tr>
<tr>
<td>4. Assessment of the neutralizing activity of vaccinated animals’ sera using variola virus to support the development of less reactogenic fourth-generation vaccines.</td>
<td>8</td>
<td>5</td>
<td>Yes</td>
<td>April 2018</td>
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<tr>
<td><strong>CDC</strong></td>
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</tr>
<tr>
<td>1. Use of live variola virus to support less reactogenic vaccine development: continued evaluation of third generation vaccines</td>
<td>10</td>
<td>3</td>
<td>Yes</td>
<td>February 2018</td>
</tr>
<tr>
<td>2. Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus</td>
<td>8</td>
<td>4</td>
<td>Yes</td>
<td>February 2018</td>
</tr>
<tr>
<td>3. Use of live variola virus to maintain and regenerate non-infectious variola virus derived materials for diagnostic development support</td>
<td>9</td>
<td>3</td>
<td>Yes</td>
<td>February 2018</td>
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<tr>
<td>4. Use of live variola virus to determine whether mice are a suitable model for human smallpox</td>
<td>7</td>
<td>6</td>
<td>Yes</td>
<td>February 2018</td>
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<td>5. Use of live variola virus to evaluate antivirals against variola</td>
<td>10</td>
<td>2</td>
<td>Yes</td>
<td>February 2018</td>
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<tr>
<td>6. Use of live variola virus for testing antiviral therapeutic</td>
<td>5</td>
<td>2</td>
<td>Yes</td>
<td>February 2019 (partial)</td>
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</table>

Study presented out of cycle (June 2018) following FDA request, recommended for approval by 5 of 7 responding ACVVR members, discussed at ACVVR 20 (Sept 2018), partially approved.
Report on the variola virus collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA

Rinat Maksyutov

Federal Budgetary Research Institution, State Research Centre of Virology and Biotechnology, VECTOR, Rospotrebnadzor, Koltsovo, Novosibirsk Region, Russian Federation

The arrangements for and experimentation with the variola virus (VARV) material in the Russian collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at the State Research Centre of Virology and Biotechnology, VECTOR, are in compliance with national and international requirements, and WHO recommendations. The conditions of storage of the virus in the repository are rigorous and secure, satisfying the applicable national and international regulations. Freezers have alarm systems and operating parameters are continuously displayed for monitoring. Electrical supplies include back-up and independent generators. Access is constantly monitored, strictly limited and regulated. For both inventory checking and research, airtight metal containers with variola virus are moved into BSL-4 laboratories located in the same building as the variola virus repository facility. In 2016, the WHO biosafety inspection team confirmed the compliance of the variola virus storage conditions with current international requirements.

Currently, the variola virus collection comprises 120 strains, originating from countries in the WHO African, Americas (countries in South America), South-east Asia, European and Eastern Mediterranean regions.

The variola virus 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 involving the use of live variola virus has been ongoing at the State Research Centre of Virology and Biotechnology, VECTOR, since 3 September 2018 and will continue in 2019, on the following topics:

(1) discovery and testing of novel chemical antiviral compounds for smallpox treatment and prevention
(2) replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development
(3) evaluation of antiviral agents against smallpox based on monoclonal antibodies
(4) assessment of the neutralizing activity of vaccinated animals’ sera using variola virus to support the development of less-reactogenic fourth-generation vaccines.
The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention in Atlanta, Georgia, continues to maintain one of two consolidated, international collections of variola virus strains. Most of these viruses were originally isolated on embryonated eggs and characterized during the final years of the intensification of the campaign to eradicate smallpox. Several sequences from these original isolates have been newly generated. These novel variola virus sequences allowed identification of unique alterations within the viral target for the only approved anti-viral compound (tecovirimat) against smallpox. The United States Food and Drug Administration has requested evaluation of tecovirimat against all the different haplotypes of the viral target gene to understand susceptibility of different viral isolates to tecovirimat. Understanding the performance of potential medical countermeasures will inform the development of effective utilization strategies to save lives should smallpox (re)emerge.

The virus collection is maintained in two separate freezers, one of which is a back-up freezer that remains largely untouched. Secure databases that meet WHO’s recommendations and the requirements of the United States Department of Select Agents and Toxins 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 pool was added to the inventory between 2017 and 2018. In the past year, WHO-approved research activities using variola virus from the inventory have focused on: (1) neutralization potential of antiviral agents (human monoclonal antibodies); (2) evaluation of sera from vaccinees receiving different vaccination regimens to evaluate efficacy based on variola virus neutralization; (3) development of protein-based diagnostics and detection assays specific for variola virus; (4) regeneration of non-infectious material for diagnostic support; and (5) determining whether humanized mice are a suitable animal model for human smallpox. The laboratory space in the high-containment facility was in active use from June 2017 through April 2018; the laboratory underwent decontamination before preventive maintenance during April and May 2018. The laboratory once more became operational on 1 June 2018.

1 In the United States of America, variola virus is a select agent and is subject to the select agent regulations (42 CFR part 73).
Use of live variola virus to maintain and regenerate non-infectious variola-derived materials for diagnostic development support

Victoria Olson, Ashley Kondas, Kimberly Wilkins, Todd Smith, Christina Hutson, Yu Li
WHO Collaborating Center for Smallpox and other Poxviruses, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

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 result in reintroduction of variola virus. The consequences of either false-negative or false-positive results will significantly impact global public health efforts. As has occurred in the recent past, new isolates of orthopoxviruses are being identified and can confound current diagnostic assays. Furthermore, “older” (vintage 1990-2000) nucleic acid diagnostic platforms are no longer being supported by some companies, necessitating development of newer platforms that must be reviewed by the US Food and Drug Administration (FDA). The need to maintain variola DNA and variola antigen stocks at the WHO Collaborating Centre for Smallpox and other Poxviruses remains important for development and validation of diagnostics. Building rapid and accurate diagnostic capacity for laboratories world-wide is crucial for successful disease containment in the event of a re-emergence of smallpox. Evidence of this occurred during the response in 2014-2016 to outbreaks of Ebola virus disease in West Africa, where the need for rapid and accurate diagnostic capacity in remote or central laboratories was critical for effective disease control.

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 as internal assay positive controls. For sensitivity analyses, use of virus DNA that has been extracted from purified virions allows a calculation of the limit of detection. Full genomic variola virus DNA will continue to be used to validate detection assays, as well as human clinical diagnostics. Over the past year we have focused on maintaining FDA regulatory approval of real-time PCR diagnostic assays by validating sensitivity and specificity as the assay is transitioned to new reagents and/or equipment as technology advances. Additionally, we have completed the optimization of combining two variola specific real-time PCR assays and one orthopoxvirus-generic quantitative PCR assay in a multiplex format, which can be used in an automated diagnostic platform. In collaboration with a commercial company, we are optimizing this assay to be used in a stable lyophilized multiplex format in the cartridge-based nucleic acid amplification test (NAAT) system. In the BSL-4 laboratory at CDC, the multiplexed diagnostic assays were able to detect both live and heat-inactivated variola virus (spiked onto a swab to simulate a clinical sample) within the automated diagnostic platform at low levels (about 27 plaque-forming units). These initial results show great promise for creating a diagnostic assay that could be used in more remote settings. NAAT platforms are becoming more prevalent worldwide and the potential exists to use such assays for monkeypox in remote regions of Democratic Republic of the Congo, collaborating with the tuberculosis surveillance programme. Understanding the performance of a similar assay for disease detection will inform the strategies for use and logistics of diagnostic assays for surveillance. Evaluation and optimization of diagnostic assays remain high priorities as public health response efforts will depend not only on rapid and accurate detection of the first smallpox case but also on identification of commonly-confused diagnoses (i.e. adverse events to vaccination) during use of medical countermeasures, should smallpox disease ever re-emerge.

1 Additional external collaborator: Michael Vickery, BioGX.
2 In the United States, variola virus is a select agent subject to the select agent regulations (42 CFR part 73).
3 The GeneXpert® system relies on semi-automated real-time polymerase chain reaction (PCR) nucleic acid amplification technology.
Replenishment of the stocks with non-infectious material, derived from live variola virus, required for diagnostics development

S.N. Shchelkunov, T.V. Tregubchak, A.A. Sergeev, A.S. Kabanov, E.V. Gavrilova, R.A. Maksyutov

Department of Genome Studies and Development of DNA Diagnostics of Poxviruses, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology, VECTOR, Rospotrebnadzor, Koltsovo, Novosibirsk Region, Russian Federation

WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA

In 2001-2003, we conducted a collaborative project together with the colleagues from the WHO Collaborating Center for Smallpox and Other Poxvirus Infections at the Center for Disease Control and Prevention, Atlanta, USA, while we tested the viability of 55 isolates of variola virus (VARV) from the Russian VARV stocks. For 21 variola virus strains from different geographical areas, the virus was grown in Vero cell culture, DNA preparations were extracted, and collections of 20 amplicons embracing the entire viral genome were produced. These DNA amplicons of each VARV strain were subjected to hydrolysis by different restriction endonucleases followed by electrophoretic separation of the subfragments prepared (RFLP analysis). Computer-assisted analysis of the data obtained made it possible to identify the phylogenetic relationships of the VARV isolates studied.

The collection of VARV DNA thus prepared was used to test diagnostic kits in development for DNA diagnosis of human pathogenic orthopoxviruses. As a first step, we developed a test kit for species-specific diagnosis of human pathogenic orthopoxviruses based on a classic variant of multiplex PCR. The test kit, Vector-MPCR-Smallpox, received marketing authorization in 2011 and was licensed for manufacture and medicinal use in the Russian Federation. The conventional PCR techniques are currently being replaced by real-time PCR assays (RT-PCR). Based on real-time PCR, we developed a method of multiplex TaqMan real-time PCR for the specific detection and differentiation of DNA from four human pathogenic orthopoxviruses in one reaction. The developed test kit, Vector-MPCRₙ-Smallpox, received marketing authorization and was licensed for manufacture and medicinal use in the Russian Federation in 2016.

In 2017, using Illumina-based next-generation sequencing (NGS) technology and the previously derived collection of VARV DNA preparations, we determined the complete genomic sequences of 24 VARV isolates and performed computer-assisted analysis of this data.

In 2018, five VARV strains maintained in the stocks at FBRI SRC VB VECTOR, Rospotrebnadzor, from geographical regions previously not studied by us, are being cultured in order to extract DNA preparations of these strains. Full-genome sequencing of extracted genomic DNAs will be performed as well as testing of the extracted DNA samples with the help of the reagent kit, Vector-MPCRₙ-Smallpox.
WHO Advisory Committee on Variola Virus Research: Report of the Twentieth Meeting

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 early detection and recognition of smallpox should a bioterror incident result in reintroduction. The consequences of false-negative or false-positive results will significantly affect global public health efforts.

CDC researchers continue to assess monoclonal antibodies (mAbs) developed in prior years for use in rapid and point-of-care diagnostic assays. Previous results demonstrated differential reactivity of some mAbs to live and gamma-irradiated variola virus (VARV). Additional studies have confirmed these findings, and those mAbs that fail to recognize live virus antigen have been eliminated from consideration for diagnostic assay design. Similarly, mAbs that only bind but do not capture virus, as would be needed for a rapid diagnostic test, have also been eliminated. By labelling antibodies with horseradish peroxidase, we determined the specificity for VARV reactivity using different combinations. The results demonstrated improved specificity with a combination of VARV-specific mAbs compared to monoclonal/polyclonal mixtures. No cross-reactivity was seen with non-variola orthopoxviruses at concentrations as high as $5 \times 10^6$ pfu/ml. We further characterized lateral flow assays to elucidate unexplained findings that crude but not purified viruses produced positive results for VARV. Using a new VARV preparation, we were able to detect purified live virus on existing lateral flow assays and minimize concerns about the suitability of sample types for assay validation.

In parallel, CDC is working with collaborators to evaluate different detection methods and different point-of-care assay formats to improve sensitivity and retain specificity. Novel field-deployable detectors for fluorescently tagged lateral flow assays have shown improvement in sensitivity by up to 2–3 orders of magnitude compared to visual detection. Other point-of-care formats, such as immunofiltration (ABICAP), have shown sensitivity down to 1000 pfu/ml with orthopoxvirus generic mAbs. Future studies will determine how well the variola virus-specific mAbs perform in these new formats. To better understand the logistic and utilization challenges involved in performing these point-of-care diagnostic assays in resource-poor areas, CDC is collaborating with partners in Democratic Republic of the Congo to use a commercial orthopoxvirus-generic lateral flow assay for detection of monkeypox virus in patient lesion samples in a very remote location. These data will provide valuable information for feasibility and important insights into how to develop strategies to enhance preparedness and response activities regarding deployment of diagnostic assays.

CDC researchers have continued to utilize a VARV-encoded protein microarray to assess antibody reactivity from serum samples and have shown that monkeypox virus infections in central and West Africa produce a profile that is unique from vaccination. Protein-based diagnostic assays have different benefits and limitations compared to nucleic acid-based diagnostic assays; each have their own role to play within smallpox preparedness activities. Protein-based diagnostic assays have the potential to be performed without the need for laboratory equipment or infrastructure. This allows for maximal flexibility in field deployment of diagnostic assays. Building rapid and accurate diagnostic capacity for use in laboratories worldwide is crucial for successful disease containment in the event of a re-emergence of smallpox. As has been seen repeatedly in outbreak-response efforts (such as during the 2014-2016 Ebola virus disease response in West Africa), the need for rapid and accurate diagnostic capacity in remote regions is essential for effective disease control.

1. In the United States of America, variola virus is a select agent and is subject to the select agent regulations (42 CFR part 73).
Discovery and testing of novel chemical antivirals and antivirals based on monoclonal antibodies for smallpox treatment and prevention

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WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA

To date, SRC VB VECTOR has screened more than 8000 chemical compounds in vitro for their anti-orthopoxviral activity, determining the 50% cytotoxic concentration (TC50 µg/ml), the 50% virus inhibition concentration (IC50 µg/ml), and the selectivity index (SI: SI = TC50/IC50).

As a result of step-by-step testing and selection of the most active compounds, first with respect to surrogate viruses (vaccinia, ectromelia, cowpox and monkeypox viruses) and then against variola virus (VARV), the most active chemical compound, NIOCH-14, was discovered and preclinical studies with NIOCH-14 were performed. Safety, pharmacokinetics and antiviral activity studies on NIOCH-14, were conducted in vivo with ectromelia virus (mousepox) and highly human pathogenic orthopoxviruses (monkeypox and variola viruses).

In experiments in animals infected with orthopoxviruses, NIOCH-14 was demonstrated to have high antiviral activity, comparable to that of tecovirimat (ST-246). A finished oral dosage form of NIOCH-14 formulated as capsules has been developed and manufactured according to Good Manufacturing Practice requirements in order to finalize preclinical studies and conduct clinical trials. A further 66 new chemically synthesized compounds have been tested against vaccinia, cowpox and ectromelia viruses in vitro. In October-November 2018, the antiviral activity of the most promising compounds against variola virus is being studied.

With regard to the development of monoclonal anti-variola antibodies, previously we had produced single-chain human antibodies against the main human-immunodominant orthopoxviral proteins encoded by the open reading frames H3L and D8 and showed their ability to neutralize the infectivity of orthopoxviruses, including that of monkeypox virus.

In 2018, using the previously obtained data and the developed system, two cell lines producing full-length human antibodies have been designed targeting orthopoxviral proteins encoded by the open reading frames H3L and D8L. Currently, testing their virus-neutralizing activity in surrogate orthopoxvirus models is underway.
Use of live variola virus to evaluate antiviral agents (monoclonal antibodies) against smallpox

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The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox re-emerges. Some historical literature has suggested that treatment with immune products from persons who have convalesced from smallpox may provide protection against the disease. Vaccinia immune globulin (VIG) is licensed by the US Food and Drug Administration (FDA) for treatment of complications from vaccination with vaccinia virus. However, VIG is in limited supply since it is no longer mass-produced. Recently, tecovirimat was the first therapeutic to be licensed by FDA for treatment of smallpox. However, the search for a second compound with a different mechanism of action continues. Monoclonal antibodies (mAbs) or cocktails thereof could be an option; in recent years, FDA has approved mAbs for the treatment of multiple conditions and pathogens.

In 2018, CDC’s efforts have focused on identifying biological therapeutics against infection with variola virus (VARV) that were previously shown to neutralize other orthopoxviruses (OPXVs) utilizing our new 12-well format plaque-reduction neutralization assays (VARV PRNT). We have continued our collaboration with the Vanderbilt Vaccine Center and focused on screening the 48 mAbs that were identified as being able to neutralize the intracellular mature virion (IMV) or extracellular enveloped virion (EV) form of an OPXV. The Vanderbilt Vaccine Center also created two different cocktails of mAbs (Mix 4 and Mix 6) that had high capacity to neutralize both forms of OPXVs across multiple species and neutralized the IMV form of VARV better than VIG. Commercial entities have also begun production of mAbs directed against OPXVs. A commercial entity has developed a mAb cocktail targeting L1 and A33 viral proteins. Single administration of this mAb cocktail provides post-exposure protection against several OPXV infections in a variety of mouse models. In a monkeypoxvirus challenge study in non-human primates, mAb cocktail treatment given in a single dose post-exposure was fully protective compared to VIG or vehicle alone. A second commercial entity has developed a cocktail consisting of three mAbs, targeting L1, B5 and A33, which provided therapeutic efficacy in multiple studies using mouse models of OPXV infection. A cocktail consisting of two of the mAbs, targeting L1 and B5, as a single-dose prophylactic treatment provided some protection to animals challenged with monkeypoxvirus intranasally.

Although the in vivo data are very promising, the ability of these mAbs to neutralize the authentic agent of smallpox is unknown. We have determined effective concentrations 50 (EC₅₀) for several of the commercially produced mAbs and mAb cocktails against both the IMV and EV forms of the variola virus using our recently optimized plaque-reduction neutralization assay. We have also completed an initial screen of mAbs from Vanderbilt Vaccine Center and determined EC₅₀ for those mAbs that neutralized the IMV form of the virus by 50%. We have completed an efficacy study evaluating the Vanderbilt Vaccine Center Mix 4 cocktail with monkeypoxvirus challenge in the prairie dog model, which serves as a proof of concept that mAb cocktails can provide protection against OPXVs. Treatment with Mix 4 provided better protection than VIG and the non-specific pox-neutralizing antibody (2D22) in terms of mortality and morbidity. Preliminary data also suggest that Mix 4 treatment decreased the amount of viral DNA detected in the tissues. We are testing these samples to determine presence of viable virus. Based on the in vitro and in vivo data, mAbs or mAb

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1 External collaborators: Iuliia Gilchuk and James Crowe, Jr, The Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Nashville, Tennessee; Jeffrey Nordstrom, MacroGenics, Inc., Rockville, Maryland; Darryl Sampey, BioFactura, Inc., Frederick, Maryland, United States of America.

2 In the United States, variola virus is a select agent, subject to the select agent regulations (42 CFR part 73).
cocktails neutralize OPXVs, including variola virus, and should be evaluated further as a potential smallpox therapeutic.

Assessment of the neutralizing activity of vaccinated animals sera using variola virus to support the development of less reactogenic fourth generation vaccines

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Discussions at the 13th meeting of the Advisory Committee on Variola Virus Research (Geneva, 31 October – 1 November, 2011) emphasized that variola virus neutralization is of great importance for the evaluation of the specific activity of investigational new smallpox vaccines that do not cause any typical skin manifestations/signs.

Accordingly, the neutralizing activity against different variola virus strains of antibodies from the sera of vaccinees constitutes the most reliable data to allow us to assess the effectiveness of protective humoral immunity the new vaccines generate, and to identify optimal application of such vaccines across various regimens and methods of vaccination.

As part of the preclinical studies of the fourth-generation smallpox vaccine, VACdelta6 developed by VECTOR, research is being conducted into its ability to generate antibodies in rabbits, mice and guinea pigs, neutralizing both vaccinia virus and variola virus in comparison with those typical of the conventional first-generation cutaneous smallpox vaccine. Assessment of the neutralizing activity of vaccinated animals’ sera against live variola virus was expected to be completed by November 2018.
Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of ‘third’-generation vaccines

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The development of new vaccines has focused on the use of attenuated vaccine strains, such as Modified Vaccinia Ankara (MVA) and LC16m8. These third-generation vaccines, however, were not tested directly for efficacy against smallpox as most were developed towards the end of the eradication era or afterwards. We have found a statistically significant difference in neutralization titres of LC16m8 vaccinees’ sera when using different target viruses (variola virus - heterologous target versus vaccinia virus - homologous target). Therefore, evaluation of the ability of sera, generated through animal or human trials with less reactogenic smallpox vaccines to neutralize mature (MV) and extracellular enveloped virus (EEV) forms of variola virus will provide a more informative surrogate measure of efficacy. The role of variola virus 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. The plaque-reduction neutralization test (PRNT), which measures the ability of immune sera to neutralize MV forms, has been used as a primary endpoint for vaccine evaluation. However, the ability of a vaccinee’s serum to neutralize EEV may be critical for vaccine efficacy since EEV is important for viral dissemination and disease pathogenesis.

At the 19th meeting of the Committee in 2017, we presented optimized methods to determine MV neutralizing capacity of sera from MVA (Modified Vaccinia Ankara) vaccinees against variola virus (VARV) to standardize the assay, remove subjectivity and increase throughput. After optimization of the assay, consistency and sensitivity results were included in the Analytical Plan and the Redevelopment Report submitted to the United States Food and Drug Administration (FDA) for approval to proceed with the proposed study design. The VARV PRNT data will be supplemental material for a non-inferiority clinical trial dataset, which demonstrated that MVA (a next-generation, non-replicating smallpox vaccine) is non-inferior to the currently licensed, replicating smallpox vaccine (ACAM2000) for neutralization of vaccinia virus. The data on VARV neutralization will provide data to support the MVA regulatory review for FDA. After the FDA approved the Analytical Plan, blinded study samples (n = 50 per vaccine (pre-vaccination and post-vaccination blood draws)) were received by CDC and tested in duplicate with the modified VARV PRNT. Data analysis is currently underway.

Given its sensitivity, reproducibility and objectivity, this PRNT was used to develop a similar 12-well EEV PRNT. This assay has been assessed with multiple operators on different days and will be used to test monoclonal antibodies as well as serum samples. Importantly, the assay will be used to assess the neutralization ability of serum from LC16m8-vaccinated individuals. As the LC16m8 vaccine strain contains a defect in the B5R gene necessary for EEV production, it will be important to confirm the ability of this vaccine to elicit EEV neutralizing antibodies. The EEV PRNT was used to test a small subset of post-vaccination serum from LC16m8 vaccinees compared to Dryvax vaccinees, a statistically significant difference between the groups was found, with LC16m8 individuals less able to neutralize the EEV form of VARV. Analysis of additional sera will confirm whether the neutralization ability of LC16m8 vaccinees’ sera is lacking against the EEV form of VARV.

1 External collaborators: Darja Schmidt and Niels Wulff, Bavarian Nordic; Yasuhiko Shinmura and Kengo Sonoda, KM Biologics Co., Ltd. (Successor of KAKETSUKEN); Masayuki Saijo, National Institute of Infectious Diseases, Japan.
2 In the United States, variola virus is a select agent subject to the select agent regulations (42 CFR part 73).
Use of live variola virus to determine whether humanized mice are a suitable animal model for human smallpox

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Historically, laboratory research efforts have tested several animal species for susceptibility to variola virus (VARV), but so far non-human primates (NHPs) are the only animals which exhibit overt illness. However, in order to induce illness in NHPs, the required infectious dose (about $1 \times 10^8$ 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, surrogate animal models of orthopoxvirus disease have been developed to evaluate the efficacy of various safer smallpox vaccines/therapeutics. These models have limitations, such as short disease incubation periods which do not resemble human smallpox, and, as a result, are suboptimal for evaluating efficacy of antivirals as true therapeutics (after symptom onset). Finding a more permissive/representative VARV animal model would facilitate testing of next-generation therapeutics. Humanized mice (hu-mice) have become an invaluable tool for modelling human biology and disease as they provide an excellent alternative to NHPs. Researchers are using them to investigate human-specific therapeutic candidates and to evaluate the safety of biologics. Moreover, hu-mice offer a unique platform for studying human haematopoiesis, viral host-pathogen interactions, and human inflammatory responses to viruses.

Mice were housed in a CDC BSL-4 Laboratory as required by WHO and the regulations of the United States Department of Select Agents and Toxins regulations. In 2015, we intranasally (IN) challenged three types of humanized female mice (BLT ($n = 8$), hu-CD34 ($n = 8$) and peripheral blood mononucleocytes (PBMC,($n = 8$)), obtained from Jackson Laboratories with VARV ($7 \times 10^3$ or $7 \times 10^5$ pfu JAP51_hrpr strain). High dose-dependent mortality was seen in the hu-CD34 and BLT mice (both dosages) beginning at day 13; PBMC mice did not have high morbidity, and only 1 of 8 (in the $7 \times 10^5$ dose group) required euthanasia before study end due to the pain scale. Large loads of viable virus were consistently harvested from tissues, regardless of mouse strain/challenge dose. Animals IN challenged with $7 \times 10^3$ or $7 \times 10^5$ pfu manifested clinical signs (for 100%, 88%, and 13% (BLT, hu-CD34 and PBMC mice, respectively)) necessitating euthanasia, with an incubation period of 13-21 days post-infection. This preliminary study found that all three mouse strains were susceptible to VARV, and subsequent pathological and immunohistochemical analysis supported infection as the cause of morbidity in mice that succumbed to disease. To confirm that study observations were due to the presence of the introduced components of a human immune system, and not due to a feature of the background nod-scid-gamma (NSG) mouse, in 2017 we evaluated whether the immunosuppressed NSG background mouse can support VARV spread using an IN challenge with $5 \times 10^4$ or $4 \times 10^6$ pfu (JAP51_hrpr strain). Three hu-NSG mice (PBMCs) were used as positive controls and challenged with the high dose inoculum. Clinical signs and weight loss were minimal/absent in the NSG mice (both inoculums) and all NSG animals were still alive at study end (21 days post-infection). Similar to the initial study, PBMC hu-mice began displaying clinical signs late in the study (about day 19) and one animal had to be euthanized (day 19) due to a clinical score of 10. Testing necropsy samples from all three PBMC mice revealed high loads of viable virus throughout tissues. In contrast, molecular analysis revealed that although there were some internal tissues positive for viral DNA, low levels of viable virus were detected in only 2 of 10 NSG mice and only at the site of inoculation (nasal tissue). This study confirmed that unlike the hu-mice, NSG mice were relatively resistant to infection and that there was not extensive viral tropism to multiple organs. Our preliminary conclusion is that there is a

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1 In the United States, variola virus is a select agent, subject to the select agent regulations (42 CFR part 73).
human immune system component that is needed for extensive viral spread, leading to clinical signs resulting in morbidity/mortality (beginning at about day 13 post-infection for hu-mice).

The findings from the 2015 study suggested that humanized mice (particularly BLT and hu-CD34) have the potential to serve as a model for smallpox disease. 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 from a VARV challenge after symptom onset (incubation period similar to human smallpox), and limit spread of virus from the inoculation site. In order to further characterize the model, we completed a dosage study with four different VARV inoculums via the IN route (1.2 x 10^3, 3.3 x 10^3, 2.2 and 0.1 pfu; 5 mice per dose) using the BLT hu-mouse strain. This strain was chosen as it has been reported that there is a detectable IgM response in BLT mice when utilizing immunization protocols. Furthermore, although IgG responses are more difficult to generate in most of these hu-mice models, modest IgG responses have been reported when using strongly immunogenic antigens and good adjuvants in BLT mice. Three animals had to be euthanized due to signs of morbidity, with more animals succumbing in the highest-dose group (n = 2 of 5 (1.2 x 10^3 pfu group) and n = 1 of 5 (2.2 pfu group)). All surviving mice were euthanized at day 42 pi. Optimization of a 96-well plaque-reduction neutralization test (PRNT) has been completed and testing of mouse serum for neutralizing antibodies is underway. In late 2018, CDC completed the serial sacrifice study with BLT hu-mice. Mice were IN challenged with 9.4 x 10^4 pfu of VARV and groups of mice (n = 4) were sacrificed on days 2, 4, 6, 9, 12 and 14 post-infection. Tissue processing and molecular analysis (from both the dosage and serial sacrifice study) are ongoing.

The dosage study and subsequent serial sacrifice study will result in better understanding and characterization of the VARV humanized mouse model. Having this model fully characterized, and results published in a peer-reviewed journal, will allow FDA to assess its usefulness. FDA does not require additional evaluation of tecovirimat or brincidofovir against live VARV in vivo but may require this information should another anti-VARV therapeutic agent be submitted or considered for licensure. By characterizing this unique animal model, the global community will have the necessary information to make an informed decision concerning its utility for testing the efficacy of any future therapeutics against VARV in vivo.
Update on the non-replicating MVA smallpox vaccine (MVA-BN® / Imvanex® / Imvamune®)

Dr Heinz Weidenthaler
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Because of fears of the re-emergence of smallpox by either intentional or accidental release of variola virus, stockpiling of smallpox vaccines has been pursued by many countries. Traditional smallpox vaccines based on replicating vaccinia viruses are highly efficacious but carry considerable side effect risk. A mass-vaccination programme with traditional replicating smallpox vaccines would require exclusion of immunocompromised subjects, pregnant women and individuals with exfoliating skin diseases. Safer strategies are therefore needed to protect against this latent smallpox threat. The non-replicating and highly-attenuated Modified Vaccinia Ankara (MVA) vaccine has undergone extensive research with more than 8800 vaccinated subjects in completed and ongoing clinical trials, including healthy and at-risk populations.

The company has recently completed a phase III non-inferiority study of MVA vaccine versus ACAM2000 vaccine, evaluating two parameters associated with protection against smallpox to demonstrate efficacy of a safer smallpox vaccine. 440 subjects were randomized 1:1 to receive either two subcutaneous doses of MVA followed by one ACAM2000 dose by scarification, at four-week intervals (Group 1) or one dose of ACAM2000 by scarification (Group 2). Co-primary endpoints were: (1) an immunological correlate of protection – demonstration of non-inferior peak serum neutralizing antibody titres (geometric mean titre, GMT) induced by MVA-BN (Group 1) compared with ACAM2000 (Group 2), and (2) a surrogate of efficacy – demonstration that prior MVA vaccination attenuated the major cutaneous lesion or “take”, measured by maximum lesion area (MLA) in mm² and the derived area attenuation ratio (AAR) after ACAM2000 scarification. Safety and reactogenicity were followed throughout the study. Both co-primary endpoints of the trial were met. Two MVA vaccinations induced peak neutralizing antibody GMT of 153.5 at day 42 compared to 79.3 at day 28 (ratio of 1.935 (95% CI: 1.562, 2.397)), reaching non-inferiority. At day 14, when ACAM2000 is typically considered to be protective, the neutralizing antibody GMT induced by a single MVA vaccination (16.2) was also non-inferior compared to ACAM2000 (16.2), with similar levels of seroconversion (90.8% Group 1; 91.8% Group 2). Median take MLAs in Groups 1 and 2 were 0.0 mm² and 76.0 mm², respectively. This resulted in an area attenuation ratio of 97.9% comparing Group 1 to 2 (95% CI: 96.6, 98.3). MVA vaccine was safe and well tolerated with better tolerability than ACAM2000.

In conclusion, based on accepted parameters of protection, MVA vaccination protects against variola infection and has an improved safety profile compared with ACAM2000. Importantly, a single vaccination with MVA was shown to induce non-inferior neutralizing antibody titres compared to ACAM2000 at 14 days, when ACAM2000 is protective against variola virus, as indicated by the vaccine take. These data indicate that MVA may be used to protect the general population, including subjects for whom vaccination with ACAM2000 may pose significant safety risks, during smallpox outbreaks.

1 MVA-BN® has been approved as IMVANEX® in Canada and IMVAMUNE® in the European Union.
Progress towards regulatory approval of tecovirimat (TPOXX®) for treatment of smallpox and other orthopoxvirus infections in humans

Dennis E Hruby

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To counter the threat of smallpox as a bioterror weapon, SIGA Technologies Inc., is developing tecovirimat (ST-246)\(^1\) for the treatment of smallpox and other human orthopoxvirus infections. For oral tecovirimat the new drug application was filed with FDA in December 2017. Following a successful meeting of the FDA Advisory Committee on 1 May 2018, which voted unanimously in favour of the application, FDA approved on 13 July 2018 tecovirimat for the treatment of smallpox. For oral administration of tecovirimat, the company has completed a single-dose ascending-dose clinical trial. No serious adverse effects were seen, and dose linearity was observed which allows an appropriate intravenous dose to be selected in order to approximate the pharmacokinetic parameters measured with the following oral dose of tecovirimat. The company is currently manufacturing regulatory registration batches of the intravenous product and conducting a bioavailability/multiple dose study in order to advance this programme towards a subsequent regulatory filing for the intravenous product.

\(^1\) This product carries the commercial name of TPOXX®
Update on the development of brincidofovir (CMX001) for treatment of smallpox and other indications

Scott A. Foster

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Brincidofovir (CMX001) is a lipid conjugate of the antiviral cidofovir, an acyclic nucleotide analogue. It retains the broad-spectrum activity of cidofovir against double-stranded DNA viruses, and lipid conjugation improves oral bioavailability, eliminates “cidofovir-like” nephrotoxicity, and enhances potency. The lipid ester linkage of brincidofovir is cleaved intracellularly to liberate cidofovir, which is then phosphorylated to produce the active metabolite, cidofovir diphosphate. The latter inhibits orthopoxvirus replication by inhibiting the activity of the viral DNA polymerase and is synergistic in vitro and in vivo with tecovirimat, which has a different mechanism of action.

Brincidofovir has demonstrated efficacy in the rabbitpox virus model and the ectromelia virus (mousepox) model, including a good laboratory practices (GLP) study rabbitpox efficacy study, in which efficacy was demonstrated when treatment was initiated up to 48 hours after the onset of fever (about five days post-infection, approximately the mid-point in disease course). The company is conducting a second GLP rabbitpox efficacy study as well as a GLP efficacy study in the mousepox model. Results of both studies are expected in 2019. The expected dose of brincidofovir for smallpox is 200 mg once a week for three weeks (or 4 mg/kg in individuals with a body weight <50 kg). At this dose and duration, brincidofovir has demonstrated acceptable safety and tolerability in healthy subjects and in immunocompromised patients. Most adverse events associated with brincidofovir reported during the first three weeks of treatment were mild gastrointestinal events and asymptomatic, reversible elevations in serum aminotransferase activities.

In addition to the indication for treatment of smallpox, brincidofovir is in clinical development for the treatment of adenovirus infections in high-risk paediatric allogeneic haemopoietic cell transplant recipients. Brincidofovir is available in tablet and liquid formulations, with an intravenous formulation also in clinical development; manufacturing has been validated at commercial scale.
Update on the “third” generation smallpox vaccine LC16M8

Yasuhiko Shinmura
Development Department, R&D Division, KM Biologics Co., Ltd., 

LC16m8 is an attenuated replication-competent vaccinia virus developed from the Lister strain by serial passaging in primary rabbit kidney cells in the 1970s. LC16m8 has low virulence and shows good protective efficacy in animal models. The LC16m8 vaccine was licensed in Japan in 1975 based on clinical data obtained during the development stage. Currently, LC16m8 vaccine is intended for emergency use and has been stockpiled in Japan since 2001 as a safeguard against potential bioterrorism with variola virus. In 2013 WHO stated that the third-generation smallpox vaccine LC16m8 should be recommended for use as a very useful and beneficial medical countermeasure against smallpox outbreak events.

The LC16m8 vaccine is a lyophilized product and has been confirmed to have high stability for long-term storage. The current approved shelf-life is four years at -20 ºC storage, however, an ongoing stability study indicates a possible extended shelf-life. In addition, we have obtained five-year stability data on LC16m8 vaccine drug substance at -80 ºC storage.

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1 Successor of Kaketsuken, the Chemo-Sero-Therapeutic Research Institute, as product manufacturer.
FDA perspective on the development and approval of smallpox medical countermeasures

Edward Cox

Director, Office of Antimicrobial Products, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, United States of America

The US Food and Drug Administration (FDA) is responsible for ensuring the safety, effectiveness, and security of medical products, including medical countermeasures (MCMs). FDA also works to help to foster the development of medical countermeasures—with the goal of facilitating the development of products that can qualify for and achieve FDA approval1—as well as facilitating timely access to medical countermeasures in the event of a public health emergency through an appropriate regulatory mechanism.

The US Government is supporting the development of smallpox medical countermeasures, including antiviral agents, vaccines and diagnostic tests. This presentation highlights the regulatory progress made since the 19th meeting of the Advisory Committee, in 2017, as well as some of the continuing challenges.

Smallpox medical countermeasures present unique and complex scientific challenges in assembling and evaluating the evidence upon which regulatory decisions are based. The historic global achievement of smallpox eradication means that there is no smallpox disease in the world. In addition, animal models that adequately represent human smallpox disease are not available.

Working closely with developers of smallpox medical countermeasures, FDA has established feasible and appropriate regulatory pathways for their approval. The focus of FDA’s interactions with medical countermeasure developers is on providing feedback on proposed studies to support clinical safety and to determine pharmacokinetics and efficacy of animal models for antiviral agents, pivotal efficacy studies and bridging studies for the attenuated virus vaccines, and requirements for the validation of diagnostic tests. In 2015, FDA issued final guidance on developing products under the Animal Rule. The guidance enhances and clarifies FDA’s expectations and recommendations on developing products under the Animal Rule based on comments received to previous draft guidance and the aggregate experience to date. In 2018, FDA issued revised draft guidance on developing drugs for the treatment or prevention of smallpox; also in 2018, it approved tecovirimat, the first drug with an indication for the treatment of smallpox.

1 The term “approval” refers to “FDA-approval, licensure, or clearance” under sections 505, 510(k), or 515 of the United States’ Federal Food, Drug, and Cosmetic Act or section 351 of the Public Health Service Act.
ANNEX 4. List of participants

MEMBERS OF THE ADVISORY COMMITTEE

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Professor Muyembe Tamfum, Director, Institut National de Recherche Biomédicale (INRB), Avenue de la Démocratie, Gombe, Kinshasa 1, Democratic Republic of the Congo

Dr 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, 155 Changbai Road, ChangPing District, Beijing 102206, China

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Dr Aïssatou Touré, Head of Immunology Unit, Institut Pasteur Dakar, 36 Av Pasteur, BP 220, Dakar, Senegal

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1 Did not attend
Dr Henda Triki, Chief, Laboratory of Clinical Virology, Institut Pasteur de Tunis, 13, Place Pasteur, BP74, 1002 Tunis Belvédère, Tunisia

Dr Zalini binti Yunus, Senior Director, Biological and Toxin Weapons Convention Nucleus, Science & Technology Research Institute for Defence (STRIDE), Ministry of Defence, Taman Bukit Mewah Fasa 9, 43000 Kajang, Selangor, Malaysia

**ADVISERS TO THE COMMITTEE**

Dr Antonio Alcami, Research Professor, Centro de Biologia Molecular Severo Ochoa, Nicolás Cabrera 1, Campus de Cantoblanco, 28049 Madrid, Spain

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Dr. Elena V. Gavrilova, Deputy Director General for Research, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECToR, Rospotrebnadzor, 630559 Koltsovo, Novosibirsk Region, Russian Federation

Dr Christina L. Hutson, Lead, Virus-Host Molecular Interactions Team (VHMI), Poxvirus and Rabies Branch (PRB), Division of High Consequence Pathogens and Pathology (DHCPP), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, N.E., Atlanta, GA 30333, United States of America

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Dr Grant McFadden, Director, Center for Immunotherapy, Vaccines and Virotherapy, Biodesign Institute, Arizona State University, 727 E Tyler Road, Tempe, AZ 85287, United States of America

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Dr Victoria Olson, Chief, Poxvirus and Rabies Branch (PRB), Division of High-Consequence Pathogens and Pathology (DHCPP), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Centers for Disease Control and Prevention (CDC), 1600 Clifton Rd. NE, Atlanta, GA 30333, United States of America

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Professor Sergey N. Shchelkunov, Head, Department of Genome Studies and Development of DNA Diagnostics of Poxviruses, Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECToR, Rospotrebnadzor, 630559 Koltsovo, Novosibirsk Region, Russian Federation

Dr David Ulaeto, Department of Biomedical Sciences, Defence Science and Technology Laboratory (Dstl), Porton Down, Salisbury SP4 0JQ, United Kingdom of Great Britain and Northern Ireland

**INVITED PRESENTERS**

Dr Olusola Aruna, Senior Public Health Advisor, Public Health England IHR Strengthening
Programme in Nigeria, British High Commission, Plot 1137, Diplomatic Drive, Central Business District Abuja, Nigeria. (Invited to present by video-link for monkeypox session)

Dr Dennis E. Hruby, Chief Scientific Officer, SIGA Technologies Inc., Suite 230 Corvallis 4575 SW Research Way, OR 97333, United States of America

Dr Scott Foster, Director of Virology, Chimerix UK Limited, 5th Fl, 6 St Andrew Street, London EC4A 3AE, United Kingdom of Great Britain and Northern Ireland

Mr Yasuhioko Shinnmura, Manager, Development Department, R&D Division, Kikuchi Research Center, KM Biologics, 1314-1 Kyokushi Kawabe Kikuchi-si, Kumamoto 869-1298, Japan

Dr Heinz Weidenthaler, Director of Pharmacovigilance, QPPV, Bavarian Nordic GmbH, Fraunhoferstrasse 13, D-82152 Martinsried, Germany

OBSERVERS

Professor David Heymann, Professor of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine; Head, Centre on Global Health Security, Chatham House, London; Chairman of Public Health England, United Kingdom of Great Britain and Northern Ireland

WORLD HEALTH ORGANIZATION

REGIONAL OFFICES

Dr Eugene Gavrilin, CDS Labnet Co-coordinator, Regional Office for Europe

HEADQUARTERS

Dr Peter Salama,1 Deputy Director-General, WHO Health Emergencies

Dr Michael Ryan,1 Assistant Director-General, WHO Health Emergencies Programme

Dr Sylvie Briand, Director, WHO Health Emergencies Programme / Infectious Hazards Management

Dr Nahoko Shindo, Manager, WHO Health Emergencies Programme / Expert Networks and Interventions

Dr Pierre Formenty, Team Leader, WHO Health Emergencies Programme / High Threat Pathogens

Dr Asheena Khalakdina, Technical Officer, WHO Health Emergencies Programme / High Threat Pathogens (Smallpox secretariat)

Dr Rosamund Lewis, Project Leader, WHO Health Emergencies Programme / High Threat Pathogens (Smallpox secretariat)

Mr Tim Nguyen, Team Leader, WHO Health Emergencies Programme / ENI

Dr Eduardo Garcia Vargas, Medical Officer, WHO Health Emergencies Programme / ENI

Dr Kazunobu Kojima, Technical Officer, WHO Health Emergencies Programme

Mrs Anne Mazur, Principal Legal Officer, Office of the Director-General, WHO

Dr Ana Maria Henao Restrepo, Medical Officer, Department of Immunization, Vaccines and Biologicals

1 Did not attend.
Mrs Rhiannon Lloyd-Jones, Consultant, WHO Health Emergencies Programme

Mr David FitzSimons, Rapporteur

NIGERIA COUNTRY OFFICE

(Attended video-link session on monkeypox, as observers)

Dr Peter Clement, WHO Health Emergencies Programme Team Leader, Nigeria

Dr Samuel Kitgakka, Infectious Hazards Management Focal Point, Nigeria

Dr Ibrahim Mamadu, Country Preparedness and International Health Regulations, Nigeria

Dhamari Naidoo, WHO Health Emergencies Programme Laboratory Technical Officer, Nigeria