

WHO Advisory Committee on Variola Virus Research

Report of the Eighteenth Meeting

Geneva, Switzerland

2–3 November 2016



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Executive Summary

The Advisory Committee on Variola Virus Research (ACVVR) held its Eighteenth meeting on 2 and 3 November 2016 at WHO Headquarters in Geneva.

ACVVR functioning

As requested by the 69th World Health Assembly in May 2016, the Advisory Committee's Terms of Reference have been refreshed and the membership has been expanded to include new members including those with specific expertise in synthetic biology and public health preparedness.

The Advisory Committee reviewed the implications for its upcoming work in light of the 69th World Health Assembly's decision to have a substantive agenda item at the 72nd World Health Assembly in May 2019 on the destruction of smallpox virus stocks. The Advisory Committee is expected to address research priorities in the interim period cognizant that the risk of smallpox re-emergence has increased as a result of the advent of synthetic biology technologies.

Reports on the virus collections

The Advisory Committee received reports on the virus collections held at the two WHO Collaborating Centres that are authorized as repositories of variola virus: the State Research Centre for Virology and Biotechnology (VECTOR), Koltsovo, Novosibirsk Region, Russian Federation, and the US Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America (USA).

Update on research

The Advisory Committee was updated on continuing research projects using live variola virus for the development of diagnostic tests, animal models, smallpox vaccines, and antiviral and therapeutic agents. Five research projects were deemed "essential public health research" using live variola virus and therefore approved in 2016 out of eight submitted.

Participants from CDC reported to the Advisory Committee on progress and challenges in the development of humanized mouse models for use in testing variola virus treatments. It was noted that CDC's research to validate a variola virus specific lateral flow assay (a diagnostic tool) was progressing, as were efforts to produce monoclonal antibodies as a possible therapeutic treatment strategy. CDC also described continuing research on the neutralizing capacity of two third generation vaccines, IMVAMUNE and LC16m8. VECTOR did not conduct research for much of 2016 due to scheduled shutdowns for decontamination and maintenance and for licensure and biennial biosafety inspections. However, in 2016 the Russian Federation had approved the use of a Russian-developed PCR multiplex real-time orthopoxvirus test kit for clinical use within the Russian Federation.

Progress was reported by the manufacturer on the development of IMVANEX/ IMVAMUNE. Although the vaccine's phase three clinical trial was still on going, the company planned to initiate new research into the vaccine's potential use as a treatment for monkeypox in high risk populations.

The manufacturer of TPOXX (Arestyr®/ ST-246), one of two antiviral treatments under development, reported that the product was edging closer to licensure. Efficacy and pharmacokinetic studies in animals had been successfully completed and all other required studies were underway for licensure application that was being prepared. Rabbitpox model studies in Brincidofovir (CMX001), a second antiviral being investigated as a treatment for smallpox, had been completed. Studies in the ectromelia model were ongoing.

The Advisory Committee also received a report from the US Food and Drug Administration on progress toward completing the studies required for the approval of various vaccines, diagnostic tests, and therapeutic medications for use with smallpox. No clear dates were provided for licensure.

The Advisory Committee was also presented with the results of orthopoxvirus research using synthetic biology. One study used bioinformatic phylogenetics to analyse the oldest known variola virus found in a Seventeenth century mummy. The other investigation described the synthesis of a horsepox virus using available commercial biotechnology.

Repository biosafety inspections

The WHO Smallpox Secretariat reported on the status of the biosafety inspection visits for the two smallpox repositories. For the current round, VECTOR's biennial biosafety inspection was completed in 2016, and CDC's was slated for May 2017.

Recommendations and observations

- The Advisory Committee strongly concurred with the Independent Advisory Group's assessment in its 2015 report on the Public Health Implications of Synthetic Biology Technology Related to Smallpox, namely that "*there will always be the potential to recreate variola virus and therefore the risk of smallpox happening again can never be eradicated.*" The Advisory Committee noted that the advent of synthetic biology means that individuals can now create viruses such as variola, given information that exists in the public domain, and thus the threat that the virus poses to public health will not be eliminated by simply destroying the virus stocks housed in the two global repositories.
- The Advisory Committee strongly recommended that definition and implementation of policy that enhanced preparedness for a possible future smallpox event was highly desirable, especially in the context of the above mentioned capabilities engendered by advances in synthetic biology. They noted, in particular, the importance of point-of-care diagnostic tests as critical in detection of disease and thereby mitigating potential mortality and morbidity. There was consensus on pursuing a research agenda that will ensure point-of-care, generic and sensitive orthopoxvirus diagnostics in the near term. The Advisory Committee acknowledged the important work that both Collaborating Centres have already done to date on developing such diagnostics.
- Advisory Committee Members retained their prior recommendation for use of live variola virus in the development of antiviral drugs. It was observed that two compounds TPOXX® and Brincidofovir were in the final stages of work in their applications for licensure. While encouraging progress was noted, it was also recognised that there remains no certainty that either candidate drugs would receive licensure. There was recognition that very effort should be made by parties involved to expedite this process.

1. Report of the WHO Secretariat - Dr Peter Salama, Dr Sylvie Briand, Dr A. Khalakdina and Mr A. Costa

The WHO Advisory Committee on Variola Virus Research met on 02 and 03 November 2016 under the chairmanship of Professor G. Smith with Dr S. Milner as Rapporteur.

1.1 Dr Sylvie Briand, Director of the Department of Infectious Hazard Management with the WHO Health Emergencies Programme, opened the meeting by introducing Dr Peter Salama, Executive Director of the new WHO Health Emergencies (WHE) Programme. Dr Salama thanked the Committee members for their commitment and long-standing service in overseeing the research on vaccines, diagnostics and antivirals for smallpox since 1999. He provided a brief background on the WHE Programme's focus areas, which include the International Health Regulations, infectious hazard management, and emergency response and preparedness for epidemics and natural disasters and conflicts. While noting that most recently the Programme has focused on Zika, yellow fever and Rift Valley fever outbreaks, he emphasized that a goal of the Programme is to build on the solid work of WHO committees such as the Advisory Committee, noting the Advisory Committee's important work, particularly with respect to diagnostics and medical countermeasures. He underscored the importance of the Advisory Committee's work stating that the Advisory Committee is at a critical point, given the WHA's impending 2019 decision to consider destruction of the variola virus. Acknowledging the added risk from synthetic biology technologies, he encouraged the Advisory Committee to make its advice to WHO specific and clear regarding the need for research on variola virus.

1.2 The Advisory Committee Members and Advisors introduced themselves. The group included three of the Committee's five new members: Dr Zalini Younus (Malaysia), Dr Aissatou Toure (Senegal) and Dr Drew Endy (United States).

1.3 Dr Sylvie Briand further clarified the questions that the Committee needed to address during the meeting, namely which research efforts should be permitted to continue to move forward and the timetable on which the research projects should conclude. She indicated that such questions were pertinent to the discussion in 2019 at the World Health Assembly concerning the destruction of the virus. She described how WHO selected and nominated new Committee members, noting that WHO strives for both geographic and gender balance in the Committee. Based on the Committee's recommendations at the 17th meeting, new members have expertise in synthetic biology, public health research, public health ethics, and biosafety and biosecurity. She clarified the role of Committee Members versus Advisors, noting that the latter had significant expertise on a range of relevant topics but did not have the decision-making authority as the Members.

1.4 Professor Smith offered remarks and led the group in remembering the life and contributions of D.A. Henderson, who had led WHO's global effort to eradicate smallpox and had served as an expert on various global committees, among the many other important roles that he had held over his long career in public health. Various Advisory Committee Members offered their personal reminiscences and memories of Dr Henderson.

1.5 Dr A. Khalakdina provided an overview of the WHO Smallpox Secretariat's activities since the previous meeting and the decision of the 69th World Health Assembly in May 2016 to take up a substantive agenda item on the destruction of smallpox at the 72nd World Health Assembly in 2019. She provided the Advisory Committee with suggested guidance on the way forward that would aid in the deliberations of the Health Assembly at that time. During the next two-and-a-half years, the Advisory Committee would continue to conduct annual meetings and WHO would submit annual progress reports to the Health Assembly. She emphasized the need for the Committee to put forward recommendations for variola virus research that were both clear and specific.

1.6 To provide additional context for the Advisory Committee's newest Members she began with an historical overview of key decision points, activities and milestones with respect to smallpox following the disease's eradication in 1980. She highlighted the World Health Assembly's decision to destroy all remaining variola stocks in 1999 as well as its subsequent decisions to temporarily retain the stocks for research purposes. She noted the formation of the Advisory Committee in 1999 to oversee all essential work with live variola virus for public health benefit and the meetings of the Advisory Group of Independent Experts in 2010 and 2013 to undertake major reviews of the products of the research efforts in an effort to allow the World Health Assembly to reach consensus on the timing of destruction of existing virus stocks.

1.7 In accordance with the World Health Assembly's decision in May 2016 to expand the Advisory Committee membership to include individuals with expertise in synthetic biology and related areas, Dr Khalakdina described the structured and deliberative process undertaken by the Secretariat. Taking into account areas of expertise and both gender and geographic balance, the Executive Director of the WHO Health Emergencies Programme had nominated the following new Advisory Committee Members: Dr. Aissatou Toure, a medical ethicist with the Institut Pasteur Dakar in Senegal; Dr. Zalini Younus, the Director of the Science & Technology Research Institute for Defence in Malaysia; and Professor Drew Endy, a synthetic biologist from Stanford University in the United States. In addition there was turnover of old Members as follows: Dr. Supamit Chunsuttiwat, a Senior Medical Officer in Thailand's Ministry of Health, replaced his Thai colleague Professor Pilaipan Puthavathana and Dr. Rinat Maksyutov, replaced Dr. Mikheev as Acting Director of VECTOR and therefore as an Advisory Committee member.

1.8 Between January and November 2016, as recommended by the Committee at its prior meeting, the “WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA” had been updated and posted on the WHO website. Dr Khalakdina reviewed the online application form to facilitate processing requests for variola virus DNA that had been developed, as requested by the Advisory Committee, and would also shortly be available via the WHO website.¹ In discussion, she noted that WHO would ensure that information submitted on the new form would align with any information that either of the two WHO Collaborating Centres at CDC in the US and at SRC VB VECTOR in the Russian Federation, respectively, would require to fulfil the request.

1.9 Mr A. Costa provided contextual background and an update on the WHO Smallpox Vaccine Emergency Stockpile. The stockpile currently consists of approximately 33.7 million doses, most of which are housed in donating countries. Switzerland houses a portion of the stockpile consisting of 300,000 doses of the newer Sanofi-Pasteur vaccine and 600,000 to approximately 2.4 million doses (when diluted) of older vaccine used during smallpox eradication. He noted that France and Japan were currently negotiating with WHO to donate additional older (France) and third generation (Japan) vaccine to the stockpile. After a hiatus over the course of the last five years, it was noted that the Netherlands’ National Institute for Public Health and the Environment (RIVM) had now resumed potency testing of stockpiled vaccine.

1.10 Mr Costa indicated that WHO had recently developed new regulatory pathways for emergency use of stockpile vaccine in the event of a smallpox outbreak together with a regulatory framework to permit deployment of the stockpile in the event of an emergency. He noted that WHO was currently collecting safety and efficacy data on the various types of vaccines pledged to or contained in the stockpile and working with potential recipient countries to increase their regulatory preparedness to receive and deploy the vaccine as well as assess medical countermeasures during any smallpox health emergency. It was noted that the need to develop such regulatory pathways stemmed from the recognition that many of the vaccines being stockpiled by countries were no longer able to be licensed in those countries nor did WHO have a mechanism for receipt and distribution of unlicensed vaccines in emergency use situations.

2. Report on research proposals submitted to WHO in 2015-2016 - Professor D. Evans

2.1. For the benefit of the Advisory Committee’s newest Members, Professor D. Evans provided a detailed description of the guidance documentation and process by which the Advisory Committee’s

¹ See WHO Smallpox website: <http://www.who.int/csr/disease/smallpox/request-form/en/>

Scientific Subcommittee reviewed proposals submitted for research with live variola virus. He reviewed the Subcommittee's membership and noted the diversity of member opinions when voting to approve or reject research proposals. He noted that the Advisory Committee and the Subcommittee's charge has been to approve research using live virus only if such research has an "essential public health benefit." Moreover, in 2013, the Advisory Committee had determined that their priority would be to continue research with antivirals but not in other areas.

2.2. Between January and November 2016, the Subcommittee received three proposals from VECTOR and five from CDC. Of these proposals, two from VECTOR and three from CDC were approved. Proposed research projects from both Repositories for the development of animal models were not approved because members questioned the relevance of these models from a regulatory perspective. Given the US Food and Drug Administration's (FDA's) Animal Rule², an animal model would be desirable to test the efficacy of antiviral candidates, however research done to date has not demonstrated sufficient progress to justify further investment. Alternatives are available to test efficacy short of using an animal model. CDC's proposal to use live variola virus to develop protein based diagnostic assays was not approved because the need for such tests was not perceived as either essential or a priority. For details of the proposals and the Subcommittee's comments see Annex 1.

2.3. In discussion, it was suggested that the Subcommittee bring recommendations concerning approval of research projects to the full Advisory Committee during the Advisory Committee's annual meeting. The full Advisory Committee could then weigh in on which research projects to recommend to the Secretariat. It was noted that this was the way in which the Advisory Committee and Subcommittee had functioned in the past but, since the Advisory Committee typically approved the Subcommittee recommendations in full, the process evolved such that the Subcommittee recommendations were forwarded directly to the Secretariat as was currently the case. It was noted that the current process also allowed for decisions concerning research to occur more frequently and flexibly. There was a discussion on rotation of the Subcommittee Members and the Secretariat was requested to update the Advisory Committee on the membership status going forward.

3. Report on the variola virus collection at the WHO Collaborating Centre Repository in Vector, Koltsovo, Novosibirsk Region, Russian Federation – Dr S. Shchelkunov

3.1. Dr S. Shchelkunov confirmed that the work on the approved research projects with live variola virus at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository of Variola Virus Strains and DNA complied with national and international requirements. The variola virus collection still consisted of 120 strains from Africa, the Americas (Brazil), South-East Asia, Europe and the eastern Mediterranean. Strains were stored in freeze-dried and frozen cultures and as

² See FDA guidance on Animal Rule (www.fda.gov/downloads/drugs/.../guidances/ucm399217.pdf)

historic scab material from patients. Storage conditions were rigorous and secure, with limited and controlled access. The WHO inspection team in 2014 confirmed that the storage conditions complied with national and international requirements. Between January and early November this year, no research with live variola virus was conducted. This was in part because VECTOR did not receive approval from WHO to proceed with selected research projects until April 2016, in part to scheduled closures at the facility for maintenance and re-licensure requirements, and in part to the WHO Biosafety Team's scheduled October 2016 visit for which the laboratory had to be fully decontaminated.

3.2. Dr Shchelkunov indicated that research concerning antivirals, assessment of the neutralizing activity of vaccine blood sera using live virus would resume in late 2016 and continue into the following year.

3.3. He indicated that in 2016 the Russian Federation had approved the use of a Russian-developed PCR multiplex real-time test kit for clinical use within the Russian Federation. The kit tests for species-specific identification of variola, monkeypox, cowpox, and vaccinia viruses. Dr Shchelkunov also stated that preclinical trials on a highly attenuated live smallpox vaccine based on vaccinia virus with directed deletion of six individual viral genes was also currently underway within the Russian Federation.

4. 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 I. Damon

4.1. Dr I. Damon reported the status in 2016 of the collection held by CDC. The BSL-4 facility was in active use from mid-2015 through March 2016. It was shut down and underwent decontamination prior to preventative maintenance in April 2016. The facility became operational in mid-June 2016 after completion of biosafety cabinet recertification and preventative maintenance.

4.2. No new variola virus seed pools were added to the inventory between 2015 and 2016. WHO-approved research activities that have utilized variola virus from the inventory within the last year have focused on maintaining and regenerating non-infectious material for diagnostic development; neutralization potential of human monoclonal antibodies; and evaluation of sera from vaccination regimens to evaluate efficacy based on variola virus neutralization.

4.3. In 2012, CDC began to evaluate the possibility of obtaining additional genomic sequence information from the historic chorioallantoic membrane-characterized variola virus isolates, as well as some of the scabs in the repository collection. Analyses indicate that the majority of the genetic

material contained in this collection is of low quality for obtaining complete and high quality variola virus genomic assembly.

4.4. Different approaches have been evaluated to enrich the DNA being used for Illumina next generation sequencing (NGS) to enable contiguous variola virus genome assembly using de novo methods. The Collaborating Centre updated its approaches to improve and validate NGS procedures (without viral propagation), which may inform efforts to facilitate genomic analyses from primary clinical specimens. Dr Damon stated that having genomic sequences of variola virus strains is valuable for archival purposes, and such information may assist in the design and validation of DNA diagnostic assays.

4.5. Based in part on successes with Nanopore sequencing in the Ebola response, the Collaborating Centre has begun to evaluate approaches for using the “MinIon” sequencer for orthopoxvirus sequencing and disease diagnosis. Dr Damon noted that optimizing and validating these newer sequencing technologies could allow the acquisition of tremendous amounts of data from historical samples, which will better prepare sequence databases and additionally allow for robust testing of PCR diagnostic assays utilized in the event of a smallpox re-emergence.

4.6. In discussion, Dr Damon indicated that the overall number of samples retained in the repositories that were either known to, or might, contain live variola virus had been reduced as a result of the genetic sequencing activities since scab material was fully destroyed. The Advisory Committee welcomed this reduction in the total number of variola virus samples. It was also noted that advances in DNA sequencing were taking place that would reduce the time and effort to generate genomic sequences of variola virus and other nucleic acids.

4.7. The Collaborating Centre’s sequenced materials were being submitted to GENBANK and 48 variola virus genomes were available there, although only a few were considered fully complete. Three to four years ago, the Collaborating Centre reviewed and destroyed most of its animal materials collections, although some of the animal samples had been kept for use in the development of clinical diagnostics. The NIH material that had been discovered in 2015 had been destroyed, as four of the materials were higher passage isolates of materials already in the Collaborating Centre’s collection, and two had been destroyed even though it was unknown whether they were represented in the CDC’s collection.

4.8. In order to illustrate the reduction in the size of virus collections, the Advisory Committee requested a detailed inventory of both CDC and VECTOR stocks together with a detailed summary of the specific collection materials that had been fully destroyed as a result of sequencing research or

otherwise prior to next years' meeting for their review. The inventories at both repositories are provided to the WHO secretariat

5. Update variola virus repositories biosafety inspection visits - Dr K. Kojima

5.1. Dr K. Kojima provided background on the inspection process for the benefit of the Advisory Committee's newest members, explaining that WHA Resolution 60.1 (2007) requires biennial inspections of both variola virus repositories. He noted that WHO appointed inspectors come from a diversity of backgrounds and possess different types of relevant expertise in order to ensure balance of continuity and "fresh eyes". Staff from the other repository are invited to participate as observers. Inspections are conducted only during periods when facilities are not in operation and have been decontaminated. During the week-long inspection, facilities are assessed on a protocol consisting of 16 elements that is based on upon the "*European Committee for Standardization (CEN) Workshop Agreement 15793*" (CWA 15793).³ Report findings are now categorized on a four-item scale ranging from "observations" that constitute largely positive remarks or issues not directly related to the inspection score, to Priority 3 findings, which are issues requiring immediate remediation.

5.2. VECTOR's most recent inspection was completed in October 2016, and CDC's is slated to take place in May 2017. WHO releases results only once both inspections in the cycle have been completed and national security redactions have been processed. Reports of the 2014-2015 inspections are now available on the WHO smallpox website.⁴

5.3. During discussion, in response to Member queries concerning the desirability of inspecting facilities when operating versus when closed down, Dr Kojima noted that this was a challenging question, and it was important to carefully consider the risk-benefit of both approaches. He noted that the former would require all inspectors to be recently vaccinated against smallpox and to have receive nationally-approved expanded training for high-containment laboratory facilities.

6. Use of live variola virus to determine whether mice are a suitable animal model for human smallpox: update on disease pathogenesis - Dr C. Hutson

6.1. Dr C. Hutson noted that historically, laboratory research efforts have tested several animal species for susceptibility to variola virus, but currently non-human primates (NHPs) are the only non-human animals that exhibit systemic rash, illness and associated mortality. However, in order to induce illness in NHPs, the required infectious dose of variola is much greater than the dose likely required for human infection. A more permissive/representative animal model system would facilitate the evaluation and understanding of next-generation, safer smallpox vaccines and therapeutics.

³ See European Committee for Standardization Workshop Agreement 15793 (http://www.uab.cat/doc/CWA15793_2011)

⁴ See the most recent safety inspection reports (<http://www.who.int/csr/disease/smallpox/safety-inspections/en/>)

6.2. Humanized mice have become an invaluable tool for modelling human biology and disease as they provide an excellent alternative to non-human primates. Such mice have been successfully used to investigate disease pathogenesis of multiple viruses that only infect human cells, including HIV, dengue virus and Epstein-Barr virus.

6.3. During 2015, CDC intranasally challenged three different types of humanized female mice (BLT, hu-CD34 and PBMC), with a range of doses of variola virus. Control mice were challenged with phosphate-buffered saline or gamma-irradiated variola virus using identical inoculation methods. High mortality was seen in the hu-CD34 and BLT mice beginning as early as day 13 post-infection. In comparison, PBMC mice had delayed morbidity/mortality. At day 21 post-infection, all surviving animals were humanely euthanized for necropsy.

6.4. Analysis of samples (heart, kidney, liver, lung, ovaries, spleen) by standard molecular and immunohistochemical pathologic assays was completed during 2016. For each mouse strain, the majority of tissues collected contained viable virus (ranging from 6×10^3 - 4×10^{10} pfu/gram). A subset of animals developed a cutaneous lesion on the hock. Post mortem analysis of hock tissue found evidence of virus (both in tissue culture and via immunohistochemistry). Hu-CD34 and BLT mice had similar pathological findings, with widespread tissue necrosis and immunohistochemical labelling of poxvirus antigen in all analysed tissues. PBMC mice had overall similar findings, with less liver and bone marrow but more lung involvement, and slightly more prominent inflammation. Poxvirus particles were seen by electron microscopy in a subset of analysed mouse tissues.

6.5. In summary, after an intranasal challenge with variola virus, the virus spread systemically before death occurred in the BLT and hu-CD34 mice beginning on day 13 post-infection. Molecular analysis (tissue culture and immunohistochemistry) of collected tissues confirmed large viral loads.

6.6. Noting that research in animal models such as this was not approved by the Advisory Committee in 2016, Dr Hutson argued that the features of the humanized mice (BLT and hu-CD34) model may make it suitable for efficacy testing of potential anti-viral therapeutics against a variola virus infection, i.e. in the ability of therapeutics to protect the mice from a lethal challenge of variola virus and stop the spread of the virus from the inoculation site. Currently there are no FDA approved antiviral compounds for treatment of poxvirus infections. Dr Hutson indicated that CDC's current mouse study suggests that the humanized mouse variola virus model would be valuable for *in vivo* testing of next-generation medical countermeasures.

6.7. In discussion, it was suggested that Dr Hutson and her team consider assessing the cytokine response in the animals in the future. Regarding the sterility of the mice and their environment, it was noted that it would be important to test whether the variola virus used in the experiments was sterile.

The Advisory Committee noted that although the humanised mouse did not precisely replicate human smallpox disease, for human smallpox, they might become useful systems in which the efficacy of anti-variola virus drugs could be evaluated.

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

7.1. Dr Damon noted that the CDC had continued to work with many colleagues to advance the development and evaluation of diagnostics. As new technologies replace older technological platforms, nucleic acid based diagnostics are constantly going through re-evaluation and revalidation. For smallpox PCR diagnostics, these efforts have largely utilized genomic variola DNA. However for protein-based diagnostics, which detect viral particles, the use of live virus or materials derived from inactivated virus particles has been critical to advance protein based diagnostic approaches.

7.2. 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. The CDC reported on the recent collaboration with the Golightly laboratory in the evaluation of a high-consequence-pathogen multiplex diagnostic, as well as updates on the smallpox diagnostic assays submitted in 2016 to the FDA for review. Such materials would continue to be used to validate detection assays, as well as human clinical diagnostics, from WHO Member States. Currently, CDC is assessing a point-of-care diagnostic real-time polymerase chain reaction assay that uses multiplex techniques and updates will be presented at the next meeting. Additionally, approaches such as the Nanopore technologies could also be used to provide diagnostic capacity for smallpox. It is likely that current technology would be useful if amplicons were initially obtained, similar to what was done in West Africa for the Ebola response by the European Mobile Laboratories.

7.3. The availability of assays that do not require stable electrical power to run the detection platform has been shown to be useful in an outbreak response situation. These methods would likely be useful for more rapid identification of orthopoxvirus or smallpox illness. For example, use of such assays may have expedited the presumptive identification of monkeypox in South Sudan in 2005 to less than three months.⁵

7.4. One method for the rapid identification of smallpox disease is detection of viral antigens by lateral flow assays. Previously, CDC published an evaluation of the Tetracore assay (a generic

⁵Damon IK, Roth CE, Chowdhary V. Discovery of monkeypox in Sudan. *New England Journal of Medicine*, 2006, 355:962-963.

orthopoxvirus detection assay) with current limited commercial availability, and not submitted to the FDA for review. CDC has been validating a variola virus specific lateral flow assay with gamma-irradiated virus. Using approaches described at the Seventeenth Advisory Committee meeting, a novel set of monoclonal antibodies with some specificity for variola virus was identified. Of four antibodies piloted so far in a lateral flow assay, positive results were seen using crude gamma-irradiated virus preps at concentrations below 1×10^6 pfu/ml. Interestingly, CDC has not yet observed reactivity with gamma-irradiated purified virus. Efforts to understand this discordance and optimization to improve sensitivity would be valuable for completion of this project. CDC has also generated a variola virus encoded protein microarray to test antibody responses against individual proteins. It was initially used to evaluate the monoclonal antibodies described in a subsequent presentation. This array approach may be informative in identification of variola infection vs. vaccination response, and additional clinical samples were being sought to evaluate this.

7.5. In discussion, the importance of a diagnostic assay that could distinguish between variola and other strains of orthopoxviruses, including monkeypox and varicella, was noted. Questions were also raised about the ability of such diagnostic tests to detect a synthetic virus.

8. Use of live variola virus to evaluate antiviral agents against smallpox - Dr V. Olson

8.1. Dr Olson provided an update on the CDC's work on antiviral therapies, noting that two small molecule compounds were in advanced development. Convalescent serum therapy has been considered and studied for the treatment of many diseases, including vaccinia infection, Ebola infection, and smallpox infection. Limited use of vaccinia immune globulin with vaccination showed additional benefit to prevent disease compared to vaccination alone amongst contacts of smallpox cases "treated" post-exposure. Many of those involved in the smallpox eradication efforts postulated that immune gamma-globulin from smallpox survivors would provide greater protection, and could be useful as a therapeutic.

8.2. Current considerations have suggested the need for two antiviral compounds, with distinct mechanisms of action, to be licensed and available for use. However, viruses are known to adapt to gain antiviral resistance, and resistant orthopoxviruses have been identified for the most advanced potential therapeutic compounds. As advances in synthetic biology allow for manipulation of viruses, it is important to remain vigilant in identification of sufficient therapeutic options should an outbreak occur. To date, there are no FDA approved antiviral compounds for treatment of poxvirus infections.

8.3. The Vanderbilt Vaccine Centre laboratory in the United States, which is collaborating with CDC, used a highly-optimized human hybridoma technology to create a large panel of monoclonal antibodies from vaccinia virus immunized persons or persons who survived either monkeypox or variola virus infection. These monoclonal antibodies were directed against multiple viral proteins,

with 48 that were capable of complement dependent neutralization of the intracellular mature virion (IMV) or external enveloped virion (EMV) forms of an orthopoxvirus. Ninety-eight per cent of neutralizing antibodies targeted one of six proteins (H3, A27, A33, B5, L1, or D8). Based on this information, two mixes (Mix4 and Mix6) were designed that had high-capacity to neutralize both forms of the virus across multiple orthopoxvirus species. The monoclonal antibody cocktails (Mix 4 and Mix 6) were protective *in vivo* within a lethal vaccinia virus challenge mouse model. These antibody mixtures neutralized variola virus *in vitro* with greater efficiency than Vaccinia Immune Globulin (VIG), indicating that the monoclonal antibody mixtures may be more efficacious than VIG for treatment of smallpox. CDC is currently evaluating the neutralizing capability of the individual monoclonal antibodies that comprised the two mixes to determine which antibody (or antibodies) conferred the neutralization against variola intracellular mature virus seen with these mixes.

8.4. Additionally, CDC is evaluating the neutralizing capability of remaining monoclonal antibodies against variola intracellular mature virion to determine if additional individual monoclonal antibodies from the panel neutralize the intracellular mature virion form of the virus more efficiently than the monoclonal antibody mixes.

8.5. CDC is also determining the neutralizing capability of these 48 individual monoclonal antibodies against the variola virus extracellular enveloped virion form, which will be critical for determining efficacy of the monoclonal antibodies as a therapeutic agent due to the importance of the extracellular enveloped virion form for dissemination and pathogenesis of the virus.

8.6. These data may suggest that a different cocktail of monoclonal antibodies would produce even more efficient neutralization against variola virus. If different monoclonal antibodies are identified with greater efficiency to neutralize variola virus individually, a variola virus specific cocktail could be created by the Vanderbilt Vaccine Centre and tested *in vitro* to determine neutralizing efficiency against the intracellular forms of the virus compared to the current monoclonal antibody cocktails.

8.7. Dr Olson noted that these cocktails show promise as a possible smallpox therapeutic strategy, noting that additional *in vivo* studies (such as with surrogate orthopoxviruses) are warranted in order to assess this potential benefit. Further, the potential exists to design strategies for use of monoclonal antibodies in therapeutic or post-exposure regimens. As research advances continue, novel therapeutic regimens (such as passive immunotherapy) should be explored.

8.8. In discussion, it was noted that CDC was exploring the use of antibodies as a treatment for variola virus infection. The value of considering comparisons of other various discrete combinations

of antibodies to discern the most effective combination for neutralizing variola virus infection was also noted. Questions were also raised concerning the manufacturing ability to produce monoclonal antibodies on a scale necessary for the research to yield an effective treatment.

9. Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of “third” generation vaccines - Dr V. Olson

9.1. Dr Olson provided an update on current research involving third generation vaccines. As background, she noted that smallpox was eradicated using traditional, “first generation”, live virus vaccines (vaccinia virus). However, the number of adverse events from universal vaccination with non-attenuated, live virus vaccines may be unacceptable today. The number of adverse events is likely to be higher than during the eradication era since a higher percentage of the current population have immunosuppressive conditions or are on immunosuppressive medications. Modified and attenuated live vaccines—“third generation” such as Modified Vaccinia Ankara (MVA)/ IMVAMUNE® and LC16m8—were developed towards the end of the eradication programme in order to address concerns about adverse events associated with the non-attenuated smallpox vaccines, but they were never tested for efficacy against smallpox.

9.2. In the absence of an animal model of smallpox disease, variola virus neutralization *in vitro* is an informative surrogate measure of smallpox vaccine efficacy. The plaque reduction neutralization test (PRNT) measures the ability of immune sera to neutralize mature virus forms and has been used as a secondary endpoint for the evaluation of new vaccines. This test has been used as a comparator and benchmark against the efficacy of the historic vaccinia virus Dryvax vaccine. Use of the PRNT is especially important when a “third” generation vaccine (such as MVA) which does not produce the classic dermatologic response (“take”), is evaluated. The “take” was the historic measure of successful vaccination. CDC has collaborated on evaluating sera from multiple “third generation” vaccine trials and found there were statistically significant differences between neutralization based on different virus antigens used in the neutralization assay.

9.3. In collaboration with the National Institute of Infectious Diseases in Tokyo, CDC has further analysed vaccinee sera from a clinical trial with LC16m8. Vaccination with LC16m8 induced an immune response capable of neutralizing variola virus. Neutralization of variola virus by sera from either vaccination regimen (LC16m8 or Dryvax) did not vary significantly. Also, both vaccination regimens caused similar percentages of participants to gain a four-fold or eight-fold rise in neutralization titer, indicative of a strong neutralization response. Consistent with previous results from other clinical trials, the efficiency of serum neutralization differed significantly based on the viral antigen used in the plaque reduction neutralization test. LC16m8 vaccinee sera neutralization of vaccinia virus-Dryvax underestimates the ability of LC16m8 vaccinee sera to neutralize variola virus-

Sierra Leone – suggesting that vaccinia virus-Dryvax neutralization assays cannot be substituted for variola virus neutralization assays. Furthermore, when neutralization of vaccinia virus-Dryvax was compared between the vaccination regimens, LC16m8 vaccinee sera was found to neutralize significantly less well compared to Dryvax vaccinee sera, which was not the case when neutralization of the causative agent of smallpox was compared. Slight differences in orthopoxvirus antigens likely account for the differences in neutralization potential between PRNT assays and underscore the need to use the virus of interest (in this case variola virus) when assessing an *in vitro* neutralization after vaccination for use as a surrogate measure of smallpox vaccine efficacy. Without an accurate measure of vaccine efficacy *in vitro*, some newer vaccines may appear to be inferior in protection from disease with the surrogate PRNT analysis.

9.4. Dr Olson indicated that CDC had also initiated discussions on how to standardize methods to determine mature virus neutralizing capacity of IMVAMUNE and extracellular envelope virus neutralizing capacity of LC16m8 vaccinee sera against variola virus. In an effort to improve upon the dynamic range and reproducibility of the traditional PRNT assay, as well as of the extracellular envelop virus PRNT assay CDC has optimized parameters of both. Such data would be important for future submissions towards regulatory approval for these “third” generation vaccines because there is currently no relevant animal model for smallpox disease in which the efficacy of these vaccines can be tested.

9.5. In discussion it was noted that IMVAMUNE®, Bavarian Nordic’s vaccine, was currently in a phase-three clinical trial, and the vaccine would likely soon be submitted to the FDA for approval. Reasons potentially underlying differences in titres among cohorts receiving Dryvax versus LC16m8 challenged with different viral strains were also discussed.

10. Gene sequencing of variola virus in a Lithuanian mummy – Professor G. Smith

10.1. Professor G. Smith presented an overview of the results of a recent gene sequencing study of variola virus found in a seventeenth century mummy.⁶ Short DNA fragments of the variola virus were found in the mummy of a Lithuanian child estimated by carbon-14 dating to have died between 1643 and 1665. Using molecular clock analysis and plotting the evolution of the variola virus on a timeline the researchers were able to show that the mummy’s virus predated all other sequenced versions of variola. Findings indicate that the time-scale of smallpox evolution is more recent than previously thought. The divergence of the virus was likely a relatively recent phenomenon and the split between major and minor strains of the virus probably occurred in the 1880s. Variola virus lineages eradicated

⁶Duggan AT, et al. 17th Century Variola Virus Reveals the Recent History of Smallpox, *Current Biology*, 2016, (in press) [http:// dx.doi.org/10.1016/j.cub.2016.10.061](http://dx.doi.org/10.1016/j.cub.2016.10.061).

during the 20th century had only been in existence for roughly 200 years, a time when human populations were both increasingly mobile and inoculation or vaccination was increasingly widespread.

10.2. In discussion it was noted that the mummy's virus, if intact and well-preserved would still be virulent and life threatening in humans today. Questions were raised about the preparation of the virus in the study. It was noted that the study provided significant insight into the variola virus' genetic stock and evolution.

11. Update on LC16m8 vaccine - Dr H. Yokote

11.1. Dr H. Yokote provided an update on the LC16m8 vaccine, illustrating in significant detail the various steps conducted at the Kaketsuken production facility in Japan in order to manufacture the vaccine. LC16m8 is a live, attenuated smallpox vaccine that has been licensed in Japan since 1975. The vaccine is intended for emergency use and is currently stockpiled by the Japanese Government for that purpose. Kaketsuken has a well-established manufacturing processes for the LC16m8 vaccine and is able to supply a certain amount of the vaccine with a high quality in a short time as necessary. The Kaketsuken facility has a production capacity of 80 million doses per.

12. Update on smallpox vaccine IMVANEX[®]/ IMVAMUNE[®] - Dr N. Samy

12.1. Dr N. Samy updated the Advisory Committee on the current status of various vaccine clinical trials that her company was conducting and planning. Bavarian Nordic's IMVANEX[®]/ IMVAMUNE[®] vaccine, also known as Modified Vaccinia Ankara Bavarian Nordic / MVA-BN[®], is designed for use in the general population, including for use among those who are immune-compromised. It is licensed in Europe for use in the general adult populations but currently has more limited licensure status in Canada and the United States.

12.2. A large, randomized open label, Phase 3 trial of the vaccine, initiated in 2014, is still on-going. The trial, which is being conducted at two United States military installations in South Korea, is designed to demonstrate the non-inferiority of the company's vaccine to ACAM2000[®] and uses different endpoints to demonstrate efficacy, immunogenicity and safety. In the discussion Dr Samy noted that recruitment for the trial had been somewhat challenging.

12.3. Bavarian Nordic is also working with CDC to conduct a study in the Democratic Republic of Congo (DRC) to investigate the potential use of the vaccine in populations at high risk for contracting monkeypox. The study population will be a group of around 1,000 Congolese health workers who will be monitored for up to two years. The study's primary objectives are to determine the number of suspected and confirmed cases of monkeypox infection among vaccinated healthcare personnel and the number of monkeypox virus exposures (e.g., personal protective equipment breaches, etc.) among

vaccinated trial participants. Study protocols have been approved by all relevant institutional ethics review boards and regulatory authorities. The start date of the study has been postponed until 2017, however, to give researchers more time to overcome various logistical and operational challenges posed by remoteness of the region where the study will take place.

12.4. Several questions concerning the vaccine's cardiac safety profile were addressed. The positive nature of striving to develop medical countermeasures against monkeypox was noted by the Advisory Committee.

13. Progress towards approval and deployment of TPOXX®/ Arestyvr® (ST-246) - Dr D. Hruby

13.1. Dr D. Hruby reported on the status of TPOXX®, previously known as Tecovirimat and ST-246®, for post-exposure treatment of orthopoxvirus infections in adults. Significant progress had been made since January 2016. Pivotal animal studies have been completed, demonstrating efficacy and pharmacokinetics in the non-human primate/monkeypox and the rabbit/rabbitpox models. These animal models are accepted by FDA as being well-characterized and adequate for evaluation of smallpox countermeasures.

13.2. TPOXX® has been evaluated in nearly 600 subjects in nine clinical trials demonstrating safety and a pharmacokinetic profile highly predictive of efficacy. Safety and tolerability studies in 420 subjects with a pharmacokinetic subset consisting of 78 subjects were recently completed. Population pharmacokinetic and pharmacokinetic dynamic models from animal studies have been triangulated with human pharmacokinetic data. They have demonstrated that human plasma exposures following dosing at 600 mg twice a day exceed the pharmacokinetic standards for efficacy, established in animal models, by multiples, yet do not approach exposure levels that may trigger safety concerns. All additional required clinical studies are in progress and the new drug application is being assembled. Large-scale manufacturing is underway and TPOXX® is currently being delivered to the United States strategic national stockpile under contract from the US government. Regulatory approval of the drug is anticipated in 2018.

13.3. Several phase one studies are on-going, including two in special populations as well as a drug-drug interaction study. The potential for weight-based dosing is also being investigated, and several human factors studies that look at whether individuals taking the medication can adequately follow directions and use sprinkle capsules as directed to achieve desired endpoints are also being conducted. An intravenous formulation of the medication is being developed and production of this formulation for use in a clinical trial will likely begin in 2017.

14. Update on the development of Brincidofovir (CMX001) for smallpox - Dr F. Gray

14.1. Dr F Gray outlined the status of the development of Brincidofovir, which inhibits replication of multiple double-stranded DNA viruses including variola virus. In vitro and in vivo studies suggest a high barrier to resistance for Brincidofovir. The drug is available in oral tablet/liquid formulations with an intravenous formulation in development. Manufacturing has been validated at commercial scale. Brincidofovir has been administered to more than 1000 patients in cytomegalovirus, adenovirus, open label and named patient/emergency investigational new drug programs, including administration among immune compromised patients, those with renal and/or hepatic impairment, adults and pediatric patients. The FDA has agreed that the rabbitpox model and the mouse ectromelia poxvirus model are acceptable to support development of the drug for treatment of smallpox. In the rabbitpox model, infected rabbits with confirmed fever, were randomized to one of five blinded treatment groups: placebo, immediate Brincidofovir, or Brincidofovir delayed by 24, 48, or 72 hours. A significant reduction in mortality was observed when Brincidofovir was initiated immediately, 24, and 48 hours after the onset of fever. Brincidofovir treatment was associated with a significant reduction in circulating infectious virus in infected rabbits, with earlier drug intervention associated with a more profound reduction. Studies are on-going in the ectromelia model to support registration for smallpox. In subset analyses from the clinical program, Brincidofovir was generally well tolerated in both adults and pediatric patients when administered at doses (200mg/week) and durations (3weeks) similar to that proposed for the treatment of smallpox.

15. FDA perspective on the development and approval of smallpox medical countermeasures - Dr L. Borio

15.1. Dr L. Borio presented an overview of FDA's perspective on the development and approval of medical countermeasures against smallpox—including various diagnostics, treatments and vaccines. Diagnostics addressed included assays to detect non-variola and variola orthopoxvirus DNA, respectively, and nucleic acid based tests. Of these, only the assay to detect non-variola orthopoxvirus DNA had been approved by FDA; the latter two remained in investigational stages.

15.2. Treatments noted included vaccinia immune globulin (intravenous), TPOXX® (Techovirimat®/ ST-246), and brincidofovir (CMX-001). Of these treatments, vaccinia immune globulin was licensed by FDA in 2005 for the treatment of complications resulting from smallpox vaccination; the latter two treatments were still considered investigational.

15.3. Vaccines discussed included: ASPV (Wetvax), which was manufactured in 1956-57; ACAM2000; and IMVANEX®/IMVAMUNE® in live, liquid frozen and freeze-dried formulations, respectively. For ASPV (Wetvax) an investigational new drug application was in effect for two formulations, and a pre-emergency use authorization had been granted for a third formulation. In

effect, this permitted the use of the vaccine during a smallpox public health emergency, should supplies of the ACAM2000 vaccine, which was licensed by the FDA in 2007, be insufficient. Although the live, liquid, frozen formulation of IMVANEX®/ IMVAMUNE® was still in the investigational stage, a pre-emergency use authorization was in place for the vaccine for use among individuals of all ages with HIV infection or atopic dermatitis. The freeze-dried formulation of the vaccine remained in the investigational stage.

15.4. It was noted that performance validation requirements are the most challenging issue for the developers of diagnostic tests. However, the FDA was cognizant of the need to balance the requirement for adequate validation studies against regulatory requirements that might be overly burdensome.

15.5. Use of the FDA's animal rule in smallpox drug development was discussed. While drug developers could rely on non-variola orthopoxvirus animal models to determine efficacy, according to a December 2011 FDA advisory committee ruling, safety assessments must still be conducted in humans. In practical terms, this meant that safety and efficacy studies would be done sequentially rather than concurrently, as is the case when all testing is performed in humans. It also meant that it is extremely challenging to conduct an adequate risk-benefit assessment of the drug, and thus properly designed clinical trials at a later date may still be required to establish whether the product(s) help or harm individuals.

16. An interactive modelling approach to assessing the role of antivirals - Dr A. Kosaraju

16.1. Dr A. Kosaraju presented to the Advisory Committee a web-based modelling tool developed by the McCain Institute for International Leadership and SIGA Technologies that permits the simulation of a smallpox attack in a major metropolitan area and incorporates both vaccination and the use of antiviral therapy in response. Dr Kosaraju's presentation was based on a model described in a paper published in the *Journal of Biosecurity and Bioterrorism* in 2013.⁷ The web-based modelling tool varies a number of key parameters, such as the size of the initial attack, the speed of government interventions, cooperation of the population with vaccination, R-nought and antiviral availability to determine the effect on three key outcomes: the mortality rates, the morbidity rates, and time to eliminate the outbreak from the population.

⁷ Finn P, Kosaraju A, Rose E, Rubin H. The role of vaccination, antiorthopoxvirus drug, and social cooperativity in a mathematical model of smallpox control. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*, 2013, 11(1):59-72.

16.2. Three different scenarios and their predicted outcomes were presented. The rationale underlying the possible selection values for the tool's key parameters was discussed. The degree of "cooperation" with vaccination according to the model has a significant impact on mortality, regardless of when the antiviral is administered.

16.3. The Advisory Committee raised a number of issues during discussion regarding methods and assumptions. It was noted that quarantine and isolation have been the main response in many epidemics, and these have been extremely effective and it was suggested that these public health measures be factored into the model as a primary response. The Advisory Committee indicated that R-nought in other models was lower than what was being used here, in part because individuals tend to isolate themselves when sick with smallpox. To further illustrate this point, the Advisory Committee also pointed out that it is not when individuals first develop a fever that they are most infectious, rather it is later when their fever goes down slightly that they become most infectious; moreover, it is after this that lesions occur. Dr. Kosaraju explained that the model is conservative in its assumptions and the R-nought was pulled from a meta-analysis of published literature where 3 is consistently the lower end of the range. Further, with an increasingly mobile population, denser cities, and unvaccinated populations, a deliberate attack would likely overwhelm isolation and quarantine effectiveness. Finally, Dr. Kosaraju mentioned that there is evidence for infectiousness at the fever stage of the disease course.

16.4. Concerns were also raised about large-scale vaccination proposed by the model, including the mortality and morbidity associated with the vaccination itself, given the potential number of immunocompromised patients and the difficulty in identifying them, in a large urban population. Dr. Kosaraju noted, however, that mass vaccination in the event of a larger attack was the CDC recommended strategy.

16.5. The importance of community engagement and communication in the event of an attack and mass exposure was also noted.

16.6. Dr. Kosaraju indicated that point-of-detection, namely number of days after the attack that smallpox would be discovered, was one factor that her team wanted to study further. She also noted that the models currently did not address post-exposure prophylaxis.

17. Discussion on criteria for approval of essential public health research

17.1. Professor D. Evans engaged Advisory Committee Members and Advisors in a discussion to gain consensus on how the Advisory Committee might clearly and narrowly define criteria for approval of essential public health research for live variola virus.

17.2. Professor Evans recalled for the group of the challenges inherent in the current research efforts that were either in-process or that were likely to be proposed. He noted that on-going research projects were often open-ended with no clear or fixed endpoints or timelines. Such studies included serum screening from earlier vaccination studies, stock maintenance and sequencing and pursuit of the next and newest technologies. With protein-based diagnostics and the use of monoclonal antibodies as a notable exception, much of the research to date had yielded only limited progress and few improvements. Moreover, some of the research may be of undetermined scientific value, as it may not be possible to ever replicate human smallpox in animal models to the degree necessary to satisfy, nor was it given that pharmaceutical companies would pursue the drug “hits” that current research efforts were uncovering. Professor Evans reminded the participants that in 2013, the Advisory Committee had determined that the group’s priority would be to approve research on antivirals for smallpox but not for diagnostics or vaccines.

17.3. The following points were highlighted by the Advisory Committee in discussion:

17.3.1. Some Members noted the positive spill over effects of current research on other orthopoxviruses such as monkeypox, which is endemic in certain areas. Others noted potential similar unintended benefits for point-of-care diagnostic technology. Others, however, reminded the group that the Advisory Committee’s charge was to focus on variola virus.

17.3.2. Advisory Committee Members acknowledged that talking further about the definition of essential research and separating the science from policy is useful. It was noted that new and emerging information concerning synthetic biology and recent knowledge gained in large public health emergencies, such as Ebola, has changed the approach to the question of what is “essential” and how medical countermeasures and diagnostic technologies are viewed.

17.3.3. The importance of maintaining a pool of individuals with sufficient expertise in different aspects of orthopoxviruses in order to create effective diagnostic and treatment interventions was noted. Whether such individuals required experience specific to variola or, more generally, to specific pathogens in order to generate effective interventions was an important question. Members acknowledged that much expertise had been brought to the table by those with orthopoxvirus experience more generally but not variola experience specifically. Similarly, it was also recognized that individuals with knowledge and expertise in mathematics, modelling and other areas of basic

science had made significant contributions with respect to smallpox diagnostics and therapeutics in the past.

17.3.4. The critical importance of research activities yielding a direct public health benefit was noted. Otherwise research activities and the resources they require would continue, with very limited public health benefit achieved from such efforts. Some Advisory Committee members questioned whether the considerable resources expended on certain variola research activities might be syphoning resources from research in other scientific areas where the public health benefit might be more clear and immediate. Several Advisory Committee Advisors, however, did not believe that this was the case.

17.3.5. It was highlighted that the Advisory Committee needed to better inform the World Health Assembly and other policy makers about why variola research required timelines beyond what the public and others might consider acceptable in order to advance the science. It was emphasized that political leaders need full information and should be made adequately aware of the context so that they can make appropriate decisions.

17.3.6. The Advisory Committee noted that the advent of synthetic biology means that it is now possible to create viruses such as variola, given full DNA sequence information that exists in the public domain. Thus the threat that variola virus poses to public health will never be eliminated by simply destroying the stocks housed in the two global repositories.

17.3.7. It was acknowledged that determining what constituted a public health benefit and creating a public health strategy with respect to variola virus that integrated biosecurity concerns was an important endeavour. It was noted that, given the advances in synthetic biology, questions stemming from the confluence of public health benefits and biosecurity concerns would arise for many diseases beyond just smallpox.

17.3.8. It was suggested that there might be value in separating the politics from the science with respect to variola. In short, the question of destroying the live virus stocks could be separated from questions concerning various types of research, their value, and their timeline. This could allow for testing of some new, yet to be discovered technology using the virus. Moreover, if entities did not want to continue pursuing research, they could stop, knowing that they could resume again in the future

17.3.9. Some Advisory Committee Members did not want to see variola virus research stopped. Rather, they wished to see it expanded. These Members acknowledged that several African countries were advocating for the destruction of the virus because the

case for longer timeframes had not been properly made. However, advances in synthetic biology coupled with outbreaks of monkeypox in some African countries meant that the public health benefit would be best served by accelerating the progress of research, particularly into diagnostics and therapeutics rather than limiting such research to two centres globally. Other Advisory Committee Members argued that this would not be useful development. Greater cooperation between the Russian and US Centres was advocated by some Members, as well as greater collaboration with other countries to further advance the field of variola and orthopoxvirus research.

18. Recreation of horsepox virus using synthetic biology - Professor D. Evans

18.1. Professor Evans presented an example of the use of synthetic biology to create horsepox virus that his laboratory recently synthesized using only commercially available information, technology and tools. The laboratory purchased DNA fragments of approximately 30 kilobase pairs (Kbp) that collectively represented the 212Kbp horsepox virus genome with short overlaps between the fragments. The short terminal hairpin loops had been ligated onto the terminal fragments to create the authentic ends of the virus genome. These DNA fragments had then been introduced collectively by transfection into cells infected with Shope fibroma virus (a Leporipoxvirus), and infectious horsepox virus had been isolated from these cells thereafter. The virus had been grown, sequenced and characterised. It had the predicted genome sequence and the growth properties described previously for horsepox virus.

18.2. The effort cost approximately 100,000 USD and took about six months. All information necessary to sequence and generate the virus was publicly available. The primary limiting factor was the length of time required by the commercial company that performed the DNA fragment synthesis.

18.3. Professor Evans' laboratory brought this activity to the attention of appropriate regulatory authorities, soliciting their approval to initiate and undertake the synthesis. It was the view of the researchers that these authorities, however, may not have fully appreciated the significance of, or potential need for, regulation or approval of any steps or services involved in the use of commercial companies performing commercial DNA synthesis, laboratory facilities, and the federal mail service to synthesize and replicate a virulent horse pathogen.

18.4. Professor Evans noted that there were various challenges involved in creating the virus but that generating an infectious orthopoxvirus pathogen was currently possible – it was no longer a hypothetical construct. In short, his demonstration of what had been predicted to be possible since 2003, when vaccinia virus was recovered from non-infectious clones of the viral genome, was a stark demonstration that this could also be done with variola virus. Moreover, recreation of such viral

genomes did not require exceptional biochemical knowledge or skills, significant funds or significant time.

18.5. The following points were highlighted by the Advisory Committee in discussion:

18.5.1. The Advisory Committee emphasized the many positive uses of this readily available technology for safeguarding and improving the public health, namely that chemical DNA synthesis means that it is now possible to make and test vaccines much more quickly as well as develop cancer-fighting oncolytics and generate greater and more rapid advances in personalized medicine. These are but two examples, among many, of the numerous ways technological advances in synthetic biology save lives and provide high-impact public health benefits.

18.5.2. The Advisory Committee acknowledged that, given the advent of synthetic biology it was no longer possible for society to entirely rid itself of the threat of smallpox or, indeed, other dangerous pathogens.

18.5.3. It was noted that the DNA synthesis technology used to make the horsepox genome depends on a chemical process that was perfected around 1980 and that requires many specialty reagents (e.g., anhydrous acetonitrile). As a result, access to long-length DNA synthesis capacities is now managed via relatively few commercial suppliers, which makes limited forms of accountability and governance possible (e.g., customer and sequence screening, record keeping). However, many academic research teams and several companies are now pursuing novel approaches to DNA synthesis and assembly (e.g., enzymatic synthesis of DNA). It was mentioned that forthcoming DNA synthesis technologies could eventually enable field-deployable (i.e., personal) DNA printers, that would make oversight frameworks that depend on screening less practical and effective. Stated differently, foreseeable next generation DNA synthesis tools are expected to be easier, quicker and cheaper than what is currently available and could become more widely available. Therefore, an important question to take up now is how to create social and political environments in which fewer individuals are enabled or incentivized to accidentally or purposefully cause harm via access to pathogens or via synthetic biology tools. How groups choose to organize themselves to address this issue may have a larger, more important impact on global public health than the technology itself.

18.5.4. Advisory Committee Members noted that by nature scientific technologies are dual-use and can thus be used for both positive and negative ends. This is true with DNA synthesis; it is also true for more basic technologies like fire. However, on balance, the

historical record has clearly demonstrated that society gains far more than it loses by harnessing and building on these scientific technologies.

18.5.5. Harkening back to the demonstrated replication of the polio virus based on information in the published literature and the level of panic surrounding this information that quickly ensued, Advisory Committee Members agreed that it was extremely important for the Advisory Committee to develop thoughtful, measured and effective strategies and messages for communicating to policy makers the nature of variola virus threats and their implication.

18.5.6. It was noted that failure to develop an effective messaging strategy concerning the synthesis of horsepox virus could limit funding for research involving synthetic biology, as had occurred following the creation of a synthetic polio virus in 2002. It was noted that while synthesis of the polio genome and recreation of the live virus may have met with serious concern in 2002, this event was quickly followed by very positive and beneficial events such as the publication in the peer reviewed literature of genomic-scale attenuation of the polio virus as a source for a safer efficacious vaccine. Subsequently, collaboration between the Venter Institute and the vaccine manufacture in the synthesis of different flu vaccine strains reduced production time required for the vaccine by several weeks, again a very positive outcome that would not have been possible without advances in synthetic biology and replication of the polio genome.

18.5.7. The Advisory Committee requested a detailed inventory of both CDC and VECTOR stocks together with a detailed summary of the specific collection materials that had been fully destroyed as a result of sequencing research or otherwise prior to next years' meetings. This has traditionally been provided by the two laboratories to the WHO Secretariat at the time of each annual ACVVR.

19. General Discussion

19.1 Research priorities for work with variola virus

19.1.1. There was a discussion of the work of the Scientific Subcommittee of Advisory Committee and the mechanism by which this group considers proposals for work with live variola virus from the two WHO Collaborating Centres. Clarity was sought on the criteria that guided decisions on whether a proposal should be approved or rejected.

19.1.2. In discussion the Advisory Committee noted the recommendation it had made in 2013, namely that work with live variola virus was no longer needed for development of

diagnostic tests and vaccines but was needed for further work on anti-viral therapeutics. Discussion also considered the length for which projects that had been approved previously should continue to be renewed, and whether further work on new animal models and early stage drug development was supportable.

19.1.3. The Advisory Committee felt that projects that had been approved previously but which were not yet complete should be allowed to continue but this could not be open-ended and proposals for extensions must specify the duration and, if approved, would be time-limited.

19.1.4. New proposals should also provide sufficient detail so that the Subcommittee could understand clearly the objectives, methods and time required. Each proposal should explain why the research was essential for public health benefit. A constructive dialogue between the two WHO Collaborating Centres and the Subcommittee was welcome.

19.1.5. Finally it was also proposed that the research projects should be submitted at a specific time of year, perhaps just a month or so before the Advisory Committee annual meeting, and the recommendations of the Subcommittee could then be considered by the Advisory Committee as a whole preceding recommendations to the WHO Smallpox Secretariat.

Diagnostic testing

19.1.6. The recent Ebola outbreak highlighted the importance of point-of-care diagnostic tests in the event of a disease outbreak. It also underscored the critical and time-sensitive role that such tests play in safeguarding the health of the public, particularly in reducing mortality and morbidity.

19.1.7. There was consensus on the importance of pursuing a research agenda that seeks to create a point-of-care, protein-based, diagnostic test that would enable rapid diagnosis of pustular rashes caused by orthopoxviruses. If a patient sample tested positive, then precautionary health care measures could be implemented rapidly. Such measures might include quarantine of the patient and vaccination of contacts, while further tests determined which orthopoxvirus was responsible for the patient's illness.

19.1.8. This point-of-care, protein-based test would likely be a dip-stick test that could distinguish orthopoxvirus infection from herpes virus (e.g. varicella zoster virus, which is commonly known as chickenpox) infections. Given the current state of technology Members believed it should be possible to create such a test in the near future.

19.1.9. In the longer term, the Advisory Committee through it desirable and possible that such point-of-care tests also have the capacity to distinguish different orthopoxviruses one from the other, i.e., the ability to distinguish variola virus from monkeypox virus, cowpox virus or vaccinia virus. However, Members indicated that the development of a test with such further capabilities should not delay the development and deployment of the aforementioned orthopox-specific test.

19.1.10. The Advisory Committee fully acknowledged the good progress that both the WHO Collaborating Centres have made to date concerning the development of nucleic acid-based and protein-based diagnostics. CDC has also gained regulatory approval for one orthopoxvirus (non-variola) DNA diagnostic assay and had submitted a variola virus specific DNA diagnostic assay for regulatory approval.

19.1.11. VECTOR has created a multiplex, real time, PCR diagnostic assay that distinguishes among variola, monkeypox, cowpox and vaccinia virus infections which has been licensed by the Russian Federation for use within that country. Another similar multiplex, real time PCR test is also in development at VECTOR that distinguishes among variola, monkeypox and varicella infections, although this test is not yet licensed.

19.1.12. CDC's advances concerning the evaluation of monoclonal antibodies that recognised variola virus antigens and thus could be used in a point-of-care diagnostic test as well as in therapeutics that neutralize variola virus were also duly noted. The monoclonal antibodies CDC was evaluating for diagnostics were derived from mice, and some showed encouraging specificity for variola virus, other monoclonals were only partially specific for variola versus other orthopoxviruses.

19.1.13. CDC expressed the view that work with live variola virus remained necessary for development of the variola virus-specific protein based test, notwithstanding the 2013 recommendation from Advisory Committee that such work did not require live variola virus.

19.1.14. Some Members of the Advisory Committee also agreed that licensure of nucleic acid-based diagnostic tests specific for variola virus was also a high priority.

19.2. Antivirals

19.2.1. The completion of experimental research on the two leading antivariola virus compounds was being prioritised and the clear time schedule for this was welcomed.

19.2.2. It was recommended that any additional work with early stage antivirals be based initially on a range of surrogate orthopoxviruses rather than just variola virus. If anti-viral compounds were identified that showed good activity against a range of orthopoxviruses (excluding variola virus) in cell culture systems and in animal models, and showed appropriate low toxicity, then proposals to develop these compounds further might be considered. It was noted that this early stage work did not need to be done at VECTOR and CDC. Rather, it could take place in other laboratories working on orthopoxviruses allowing the highly specialized staff at the two WHO Collaborating Centers to focus on live variola virus research.

19.2.3. There had been a constructive dialogue between the FDA and companies evaluating the two leading antiviral candidate compounds for variola virus. This dialogue had resulted in definition of the remaining experimental work with animal models of orthopoxvirus infection that was needed prior to submission of applications to FDA for licensure of these compounds. With these compounds, it was noted that such experimental work did not include use of live variola virus.

19.2.4. Further, a time schedule for completion of this experimental work was presented and good progress was underway. It was noted that an application for licensure of TPOXX® (formerly known as ST-246) would likely be submitted to the FDA by the end of 2017 for consideration in 2018. While the encouraging progress was noted, it was also recognised that there remains no certainty that either candidate drugs would receive licensure. This uncertainty was greater for the second lead compound, Brincidofovir (CMX001), about which some concerns and reservations were voiced.

19.2.5. All other potential antiviral drugs for smallpox were at a much earlier stage of development, and many years of work and investment would be necessary if one or more of these compounds were to be developed and licensed.

19.3. Vaccines

19.3.1. It was noted that all new vaccine research currently involved benchmarking the new vaccines against first generation smallpox vaccines. At present, there are no recommendations on the duration of such follow-up or benchmarking for those being vaccinated. The Advisory Committee recommended that on-going vaccine trials involving variola neutralization tests should be completed according to their current protocols. Moving forward, however, the Advisory Committee recommended that new vaccine trials use single or multiple endpoints within a short time frame when assessing the longitudinal stability of antibody titre.

19.4. Synthetic biology

19.4.1. The Advisory Committee emphasized that the degree to which synthetic biology has—and will continue to — change the landscape concerning variola virus cannot be overstated. The Advisory Committee strongly concurred with the WHO Scientific Working Group (SWG) on Synthetic Biology Technology’s conclusions noted in the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox’s 2015 report to the WHO Director-General. In this report, the SWG stated: “With the development of [synthetic biology] technologies, public health agencies have to be aware that henceforth there will always be the potential to recreate variola virus and therefore, the risk of smallpox re-emerging can never be fully eradicated.”⁸

19.4.2. The Advisory Committee, however, was equally firm in emphasizing that historically the impact of synthetic biology such as DNA synthesis has been to improve the public health response to disease.

19.4.3. In view of the developments in synthetic biology the Advisory Committee wished to counsel WHO that the potential sources of variola virus can no longer be considered to be restricted to the known stocks of virus in the two WHO Collaborating Centres and that it is possible now, and henceforth, that infectious variola virus could be re-created outside these centres. Although this is not a trivial task and would take a determined and sustained effort that would not happen by accident, nevertheless it could be done. The risk of the virus escaping from these highly secure and constantly monitored and inspected laboratories was considered remote, whilst the potential of virus to re-emerge elsewhere would grow, as the ease and speed of synthesizing DNA increased and the cost decreased.

19.5. Preparedness

19.5.1. Advisory Committee strongly recommended that definition and implementation of policy that enhanced preparedness for a possible future smallpox event was highly desirable, especially in the context of the above mentioned capabilities engendered by advances in synthetic biology. Such policy would include the deployment of point-of-care, protein-based tests for orthopoxviruses; the deployment of PCR-based diagnostic

⁸ See page 5 of the IAG on Public Health Implications of Synthetic Biology Technology Related to Smallpox’s June 2015 report to the WHO Director General (<http://www.who.int/csr/resources/publications/smallpox/synthetic-biology-technology-smallpox/en/>)

tests for variola virus in specialist laboratories nationally or regionally; the training of staff to recognise smallpox and distinguish this from chicken pox; and the development of generic policy that would deal with public health issues associated with a confirmed outbreak. Such public health measures might well be generic and could be applied to other outbreak scenarios. In this regard the Advisory Committee Members noted the experience in the recent Ebola virus epidemic in Western Africa has proven relevant to considering approaches to potential smallpox outbreaks.

20. Closing and next steps

20.1. In closing, the Chairman thanked the Advisory Committee Members for an animated, highly productive and illuminating discussion.

20.2. The increasing number of publications in peer-reviewed scientific journals of research undertaken at the two WHO Collaborating Centers under the auspices of the Advisory Committee research was recognized. This not only emphasised the good quality of the work undertaken but also disseminated this work openly throughout the world for all to read, evaluate and consider the implications. It was requested that CDC and VECTOR provide the Advisory Committee with a glossary of peer reviewed publications that had resulted from their research on variola virus over the course of the two decades since Advisory Committee was founded. This and all future lists will be attached as an annex to the annual report of the Advisory Committee.

20.3. It was also requested that the Subcommittee provide an update on current Subcommittee members and their tenure.

20.4. Finally, the Advisory Committee requested a detailed inventory of both CDC and VECTOR stocks together with a detailed summary of the specific variola virus collection materials that had been fully destroyed as a result of sequencing research or otherwise prior to next years' meeting. These are traditionally provided on CD-ROM to the WHO Secretariat annually.

ANNEX 1. Research proposals submitted to WHO in 2015-2016

Proponent and Projects	Y	N	Majority Opinion and Notes	
VECTOR				
1. Animal models (continuation) 2016	0	7	No	Significant doubts were expressed about the model, not seen as essential from a regulatory testing perspective
2. Antivirals (continuation) 2016	4	3	Yes	The minority still questioned the need for more work in the area given two existing drugs
3. Neutralization (continuation) 2016	7	0	Yes	Serum screening approved
Center for Disease Control				
1. Use of live VARV to determine whether mice are suitable model for human smallpox	2	5	No	Significant doubts were expressed about the model, not seen as essential from a regulatory testing perspective
2. Use of live VARV to develop protein based diagnostic assays	2	5	No	Need for additional tests was not viewed as a priority or essential
3. Use of live VARV to evaluate antivirals against VARV - monoclonals	5	2	Yes	A continuation of mAb research approved
4. Use of live VARV to maintain and regenerate non-infectious materials	7	0	Yes	Stock maintenance and sequencing approved
5. Use of live VARV to support less reactogenic vaccine development	5	2	Yes	Serum screening approved

ANNEX 2. Abstracts of presentations

REPORT ON THE VARIOLA VIRUS COLLECTION AT THE WHO COLLABORATING CENTER FOR ORTHOPOXVIRUS DIAGNOSIS AND REPOSITORY FOR VARIOLA VIRUS STRAINS AND DNA AT FBRI SRC VB VECTOR, ROSPOTREBNADZOR

S. Shchelkunov

Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor), Koltsovo, Novosibirsk region, 630559, Russia

Organization of and experimentation with the Russian variola virus (VARV) collection at the WHO Collaborating Centre (WHOCC) for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at FBRI SRC VB VECTOR, Rospotrebnadzor, is in compliance with national and international requirements, and the WHO recommendations. Instructions regulating research, as well as all maintenance and control procedures, have been developed on the basis of the documents listed above. Plans for disease control measures and response to accidents have been developed. Emergency response teams have been established for deployment in the event of accidents or emergencies.

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

In 2016, Russian government registration of the test kit for species-specific identification of variola, monkeypox, cowpox, and vaccinia viruses was obtained, which is based on real-time multiplex PCR.

Preclinical trials on a highly attenuated live smallpox vaccine, based on vaccinia virus with directed deletion of six individual viral genes, are underway.

Research using live variola virus is going to be continued in 2016 - 2017 in the following directions:

- 1. Discovery of new antivirals for smallpox treatment and prevention.*
- 2. Assessment of the neutralizing activity of vaccinee blood sera using live variola virus.*
- 3. Development of animal models to study the efficacy of therapeutic and preventive products against smallpox.*

THE WHO COLLABORATING CENTER FOR SMALLPOX AND OTHER POXVIRUSES AT THE CENTERS FOR DISEASE CONTROL AND PREVENTION ATLANTA, GA: 2015 REPORT ON THE VARIOLA COLLECTION

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The World Health Organization (WHO) Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention in Atlanta, GA continues to maintain one of two consolidated, international collections of *Variola virus* strains. The majority of these viruses were originally isolated on embryonated eggs and characterized during the final years of the intensification of the smallpox eradication campaign. The virus collection is maintained in two separate freezers, one of which is a back-up freezer which has remained largely untouched. Secure databases, which address WHO recommendations as well as U.S. Select Agent requirements have been constructed to track usage of *Variola virus*. Annual reports on the status of these collections are provided to WHO. No new *Variola virus* seed pools were added to the inventory between 2015 and 2016. WHO-approved research activities which have utilized *Variola virus* from the inventory within the last year have focused on: maintaining and regenerating non-infectious material for diagnostic development, neutralization potential of human monoclonal antibodies, and evaluation of sera from vaccination regimens to evaluate efficacy based on *Variola virus* neutralization. The laboratory space was in active use from July 23, 2015 through late March 2016; the laboratory underwent decontamination prior to preventative maintenance in April 2016. The laboratory once more became operational in mid-June 2016. In the United States, *Variola virus* is a select agent and is subject to the select agent regulations (42 CFR part 73).

USE OF LIVE VARIOLA VIRUS TO DETERMINE WHETHER MICE ARE A SUITABLE ANIMAL MODEL FOR HUMAN SMALLPOX

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A number of animal models of systemic *Orthopoxvirus* disease have been developed to evaluate various safer smallpox vaccines or therapeutics. Many of these animal models have limitations, such as short disease incubation periods, which do not resemble human smallpox. As a result, these systems can be difficult to use to evaluate the efficacy of antivirals as true therapeutics (i.e. after the onset of symptoms) or to evaluate the post-exposure prophylactic use of newer smallpox vaccines. During the eradication campaign, post-exposure vaccine use was a critical component in disease control and ultimate disease elimination.

In the United States, *Variola virus* is a select agent and is subject to the select agent regulations (42 CFR part 73). Historically, laboratory research efforts have tested several animal species for susceptibility to *Variola virus*, but as yet, non-human primates (NHPs) are the only non-human animals which exhibit overt illness. However, in order to induce illness, the required infectious dose is much greater than the dose required for a natural infection (1×10^8 to 1×10^9 *Variola virus* virions). The discovery of a novel, more permissive/representative animal model system would facilitate the development of next-generation, safer smallpox vaccines and therapeutics.

Humanized mice have become an invaluable tool for modeling human biology and disease as they provide an excellent alternative to NHPs. Researchers are using them to investigate human-specific therapeutic candidates and evaluate the safety of biologics. Moreover, humanized mice offer a unique platform for studying human hematopoiesis, viral host-pathogen interactions, and human inflammatory responses to viruses. These animals have been successfully used to investigate disease pathogenesis of multiple viruses which only infect human cells, including HIV, Dengue virus and Epstein- Barr virus, which supports the evaluation of these humanized mice as an animal model of the solely human pathogen *Variola virus*.

Under the approved proposal, in 2015 we intranasally challenged three different types of humanized female mice (BLT, hu-CD34 and PBMC), obtained from Jackson Laboratories (Bar Harbor, ME) with a range of doses of *Variola virus* [5×10^5 or 7×10^7 plaque forming units (pfu)]. Control mice were challenged with PBS or gamma-irradiated *Variola virus* using identical inoculation methods. Mice were co-housed in groups of 2-4 animals per ventilated cage in the High Containment Laboratory (BSL 4). Sterile mouse husbandry practices were performed during the experiment in accordance with CDC Institutional Animal Care and Use Committee (IACUC) guidelines. Daily observations of the animals' food consumption, activity level, and general appearance were recorded. Mice were also monitored for clinical criteria warranting euthanasia. Three times per week body weights were recorded, complete skin exams were performed, and oral swabs were collected while under anesthesia with 3-5% of isoflurane gas. High mortality was seen in the hu-CD34 and BLT mice (both the 5×10^5 and 7×10^7 pfu challenged groups) beginning as early as day 13 post infection. In comparison, PBMC mice had delayed morbidity/mortality. At day 21 post infection, all surviving animals were humanely euthanized for necropsy. Samples were analyzed by standard molecular assays. For each mouse strain, the majority of tissues collected contained viable virus (range from 6×10^3 - 4×10^{10} pfu/gram). Hu-CD34 and BLT mice had similar pathological findings, with widespread tissue necrosis and immunohistochemistry labeling of poxvirus antigen in all analyzed tissues. PBMC mice had overall similar findings, with less liver and bone marrow and more lung involvement, and slightly more prominent inflammation. Preliminary results were presented at the January 2016 ACVVR meeting and final molecular analysis results were presented during the November 2016 meeting.

USE OF LIVE VARIOLA VIRUS TO MAINTAIN AND REGENERATE NON-INFECTIOUS VARIOLA DERIVED MATERIALS FOR DIAGNOSTIC DEVELOPMENT SUPPORT

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The ability to validate nucleic acid-based and protein based diagnostic capacity is critical for early detection and recognition of smallpox should a bioterror incident result in reintroduction. The consequences of either false negatives, or false positives, will significantly impact global public health. “Older” (vintage 1990-2000) nucleic acid diagnostic platforms are no longer being supported by some companies, as newer platforms are developed, and have been reviewed by the US Food and Drug Administration (FDA). The need to maintain variola DNA and variola antigen stocks at the WHO Collaborating Centre (WHO CC) for Smallpox and other Poxvirus Infections remains important for future diagnostic development and validation. In the United States, *Variola virus* is a select agent and is subject to the select agent regulations (42 CFR part 73). Building rapid and accurate diagnostic capacity for use in laboratories world-wide is critical for successful disease containment in the event of a reemergence of smallpox. Evidence of this occurred during the 2014-2015 Ebola response in West Africa, the need for rapid and accurate diagnostic capacity in remote or central laboratories was critical for effective disease control. This presentation will update on our results from utilization of non-infectious *Variola virus* to validate several diagnostic assays.

DNA diagnostics

Variola virus DNA stocks are important for future diagnostic research and for our role as a WHO CC for Smallpox and other Poxvirus Infections. *Variola virus* DNA stocks are required to evaluate nucleic acid-based diagnostic assays and for training purposes such as workshops held at the WHO CC for U.S. scientists, international scientists, and the Global Health Security Action Group (GHSAG). For these purposes, stocks of *Variola virus* DNA are needed for continued diagnostic validation and future collaborative exercises with worldwide diagnostic laboratory partners. 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 (LoD). Such materials will continue to be used to validate detection assays, as well as human clinical diagnostics, from WHO member countries. Currently we are assessing a point-of-care diagnostic real-time PCR assay that uses multiplex techniques and updates will be presented.

Protein diagnostics

Since late 2011, studies have continued on monoclonal antibody characterization, viral antigen capture assays, protein microarray development and novel methods for high throughput viral neutralization assays applicable to *Variola virus*. The availability of more advanced diagnostics requiring expensive assay platforms and extensive infrastructure is not feasible in resource poor areas. One method for the rapid identification of smallpox disease is detection of viral antigens by lateral flow, which will provide valuable detection applications should smallpox ever reemerge. This past year, we have been validating a *Variola virus* specific lateral flow assay with gamma-irradiated virus. Of 4 antibodies piloted so far in a LFA assay, positive results were seen using crude gamma-irradiated virus preps at concentrations below 1×10^6 pfu/ml. Interestingly, we have not yet observed reactivity with gamma-irradiated purified virus. Efforts to understand this discordance and to begin optimization to improve sensitivity would be valuable for completion of this project. We have also generated a *Variola virus* encoded protein microarray to test antibody responses against individual proteins. The lateral flow and microarray assay updates will be presented.

USE OF LIVE VARIOLA VIRUS TO EVALUATE MONOCLONAL ANTIVIRALS AGAINST VARIOLA VIRUS

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The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox somehow re-emerges. In the United States, *Variola virus* is a select agent and is subject to the select agent regulations (42 CFR part 73). The development of antiviral strategies may be important in outbreak response efforts as well as in disease treatment. Current considerations have suggested the need for two antiviral compounds, with discrete mechanisms of action, to be licensed and available for use. To date, there are no Food and Drug Administration (FDA) approved antiviral compounds or monoclonal antibodies (mAbs) for treatment of *Poxvirus* infections. Vaccinia Immune Globulin (VIG) is licensed by the FDA for treatment of complications from vaccination with *Vaccinia virus*. However, VIG is in limited supply since it is no longer mass produced after eradication of smallpox. There is no data which supports the use of VIG as a standalone antibody prophylaxis against smallpox. There is also some historic literature which suggests that treatment with immune products from persons who have convalesced from smallpox may provide protection against smallpox.

Research within the Vanderbilt Vaccine Center laboratory have utilized a highly-optimized human hybridoma technology to create a large panel of mAbs from *Vaccinia virus* immunized persons or persons who survived either *Monkeypox virus* or *Variola virus* infection. These mAbs were directed against multiple viral proteins, with D8 and B5 being the most prevalent. Further *in vitro* characterization of the mAbs identified 48 were capable of complement dependent neutralization of the intracellular mature virion (IMV) or external enveloped virion (EEV) form of an *orthopoxvirus* (*Cowpox virus*, *Monkeypox virus*, *Vaccinia virus*). Based on this information, two mixes (Mix4 and Mix6) were designed that had high-capacity to neutralize both forms of the virus across multiple species. Most intriguing, when Mix6 was used as a therapy, it was found to provide complete protection of mice against a lethal respiratory challenge with *Vaccinia virus*. This presentation will update our progress on determining the capability of these mAbs to neutralize *Variola virus in vitro* using our classic plaque reduction neutralization assay as well as their ability to limit virus spread. Mix6 and Mix4 had greater capacity to neutralize *Variola virus* than did VIG. These mAb cocktails show promise as a possible smallpox therapeutic strategy – additional *in vivo* studies (such as with surrogate Orthopoxviruses) are warranted in order to assess benefit. Further, the potential exists to design strategies for use of mAbs in therapeutic or post-exposure regimens.

USE OF LIVE VARIOLA VIRUS TO SUPPORT LESS REACTOGENIC VACCINE DEVELOPMENT: CONTINUED EVALUATION OF “THIRD” GENERATION VACCINES

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Variola virus neutralization *in vitro* remains an informative surrogate measure of smallpox vaccine efficacy. Our prior studies, using sera from *Vaccinia virus*-vaccinees (MVA (IMVAMUNE) or Dryvax), have indicated that neutralization endpoint titers may differ when using different target viruses. Slight differences in *Orthopoxvirus* antigens likely account for these differences. Furthermore, our data demonstrated that vaccinee sera neutralized different strains of *Variola virus* with different levels of efficiency. The plaque reduction neutralization test (PRNT), which measures the ability of immune sera to neutralize mature virus forms (MV), has been used as a primary endpoint for the evaluation of vaccines. However, the ability of a vaccinee’s serum to neutralize the extracellular enveloped virus (EEV) form of virus may be critical for vaccine efficacy since EEV is important for viral dissemination and ultimate disease pathogenesis [Smith et al. J. Gen. Virol. 2002, 83: 2915-31].

The development of new vaccines has included significant focus on the use of attenuated vaccine strains, such as Modified Vaccinia Ankara (MVA) and LC16m8. These “third generation” vaccines, however, were never tested directly for efficacy against smallpox during the eradication campaign since most were developed towards the end of that era. Evaluation of the ability of sera, generated through animal or human trials with less reactogenic smallpox vaccines, to neutralize MV and EEV forms of *Variola virus* will provide a surrogate measure of efficacy. The role of *Variola virus* neutralization as a surrogate marker for vaccine efficacy is particularly valuable for the evaluation of vaccines that do not elicit a “take”, the traditional measure of vaccine success.

This presentation will update results from PRNT optimization efforts as well as collaborations with the Japanese government and industry scientists to evaluate LC16m8 vaccination regimens for *Variola virus* MV neutralization. In particular, neutralizing capacity of vaccinee sera will be compared between different *Orthopoxviruses* (*Vaccinia virus* versus *Variola virus*), and different vaccines (Dryvax versus LC16m8). We have found a statistically significant difference in neutralization titers of LC16m8 vaccinee’s serum when using different target viruses (*Variola virus* - heterologous target versus *Vaccinia virus* - homologous target). These results underscore the need to use the virus of interest when assessing *in vitro* neutralization after vaccination. We have also initiated discussions on how to standardize methods to determine MV neutralizing capacity of MVA-BN® (Modified Vaccinia Ankara - Bavarian Nordic) and EEV neutralizing capacity of LC16m8 vaccinee sera against *Variola virus*. In an effort to improve upon the dynamic range and reproducibility of the traditional PRNT assay, as well as of the EEV PRNT assay, we have optimized parameters of both. Criteria that we have evaluated include cell density seeding, utilization of an overlay, and 12 vs. 24-well formats. Additionally, for both the IMV PRNT and EEV PRNT, we have evaluated an automated plaque counter which greatly reduces subjectivity and improves upon quality control measures. We have tested the assays for reproducibility, both between technicians and at different time points and results of our optimization efforts will be presented. These data will be essential for future submissions towards regulatory approval for these “third” generation vaccines. In the United States, *Variola virus* is a select agent and is subject to the select agent regulations (42 CFR part 73).

UPDATE ON A 3RD GENERATION SMALLPOX VACCINE LC16M8

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In the 1970's, an attenuated replication-competent vaccinia virus, the LC16m8 strain, was developed from the Lister strain by serial passaging in primary rabbit kidney cells at a low temperature, as a result of efforts to establish a safer strain.

KAKETSUKEN constructed a drug substance manufacturing facility complying with J-GMP and US-cGMP for a stable supply.

Currently, the LC16m8 vaccine is intended for an emergent use. KAKETSUKEN has a well-established manufacturing processes for the LC16m8 vaccine and is able to supply a certain amount of the vaccine with a high quality in a short time as necessary.

This facility, which has been in operation since then, has a production capacity of 80 million doses per year.

UPDATE ON THE NON-REPLICATING SMALLPOX VACCINE IMVANEX[®] (IMVAMUNE[®])

N. Samy

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Modified Vaccinia Ankara (MVA-BN[®]; trade name IMVAMUNE[®] outside EU, invented name IMVANEX[®] in the EU) is a live, highly attenuated non-replicating smallpox vaccine currently stockpiled by the US Government under a pre-emergency use authorization for potential use in all individuals infected with human immunodeficiency virus (HIV) or living with atopic dermatitis, following a declared smallpox emergency. Furthermore, MVA-BN is licensed in the EU under the trade name IMVANEX and in Canada under the trade name IMVAMUNE and has also been added to the WHO stockpile as a non-replicating smallpox vaccine.

Due to the non-replicating nature and the subcutaneous administration route of IMVANEX (IMVAMUNE), traditional measures of efficacy (i.e. formation of a “take” around Day 6 as observed after scarification with replication competent smallpox vaccines) are not suitable, requiring alternative strategies to demonstrate protective efficacy. The growing preclinical and clinical database has consistently and repeatedly demonstrated that IMVANEX (IMVAMUNE) leads to comparable protective efficacy (in animal models) and immune responses as seen with traditional, replicating smallpox vaccines while holding a substantially better safety profile. Based on the current safety data generated in 19 completed trials with more than 7,500 vaccinated subjects, including healthy populations (age range 18-80 years) and people contraindicated to receive replicating smallpox vaccines (such as HIV infected subjects and individuals with a history of or active atopic dermatitis), IMVANEX (IMVAMUNE) has shown to be safe and well tolerated and does not implicate a risk of inflammatory cardiac disease as observed with conventional, replicating smallpox vaccines like Dryvax[®] and ACAM2000[®].

Currently, the CDC is preparing to initiate a clinical trial with IMVAMUNE in health care workers at risk for exposure to monkeypox. The trial is planned in an area in the Democratic Republic of Congo with a high incidence of monkeypox virus infections in humans. This is the first clinical trial to evaluate the effectiveness of IMVAMUNE against orthopox viruses in humans in an open-label, prospective cohort study.

The pathway for licensure of IMVAMUNE in the US will be based on a currently ongoing Phase 3 non-inferiority trial that directly compares indicators of efficacy of IMVAMUNE (vaccinia-specific PRNT antibody responses and attenuation of the “take” by vaccination with IMVAMUNE prior to administration of ACAM2000) to the replicating smallpox vaccine ACAM2000. A freeze-dried formulation of IMVAMUNE has shown to elicit vaccinia-specific antibody responses that are non-inferior to those induced by the current liquid frozen formulation, which will allow using the pivotal Phase 3 trial to support regulatory evaluation of safety and efficacy for both, the liquid-frozen and freeze-dried formulation.

In conclusion, the non-replicating smallpox vaccine IMVANEX (IMVAMUNE) is believed to be the most suitable candidate for being included in pre- and post-event smallpox preparedness plans, in particular for protection of first line responders, military personnel and populations with increased rates of severe side effects from replicating vaccines, such as immunocompromised and eczema patients.

PROGRESS TOWARDS REGULATORY APPROVAL OF TPOXX® FOR THE TREATMENT OF SMALLPOX AND OTHER ORTHOPOXVIRUS INFECTIONS IN HUMANS

D.E. Hruby

SIGA Technologies, Inc., Suite 110, 4575 SW Research Way, Corvallis, Oregon, 97333, United States of America.

To counter the threat of smallpox as a bioterror weapon, SIGA Technologies is developing TPOXX® (tecovirimat/ST-246) as a self-administered oral drug for the treatment of smallpox and other human orthopoxvirus infections. Because TPOXX® efficacy cannot be tested in humans due to ethical concerns and lack of naturally-occurring smallpox patients, TPOXX® efficacy has been demonstrated in numerous animal models under the auspices of the Animal Rule (21 CFR Parts 314 and 601). In particular, pivotal studies have been completed, demonstrating efficacy and evaluating pharmacokinetics in the non-human primate/monkeypox and the rabbit/rabbitpox models, which are accepted by FDA as being well-characterized and adequate for evaluation of smallpox countermeasures. TPOXX® has been evaluated in nearly 600 subjects in 9 clinical trials demonstrating exceptional safety and a pharmacokinetic profile highly predictive of efficacy. SIGA has recently completed its pivotal safety and tolerability study in 420 subjects with a PK subset consisting of 78 subjects. Population pharmacokinetic and pharmacokinetic/pharmacodynamic models from pivotal animal studies have been triangulated with human pharmacokinetic data demonstrating that human plasma exposures following dosing at 600 mg twice a day exceed the pharmacokinetic standards for efficacy, established in animal models, by multiples, yet do not approach exposure levels that may trigger safety concerns. Large-scale GMP manufacturing is underway and TPOXX® is currently being delivered to the SNS under contract from the US government. Regulatory approval is anticipated in 2018.

UPDATE ON THE DEVELOPMENT OF BRINCIDOFOVIR FOR SMALLPOX

D. F. Gray

Chimerix In., Durham, North Carolina, United States of America

Brincidofovir (BCV) is a potent in vitro inhibitor of multiple dsDNA viruses including adenovirus, cytomegalovirus and variola virus (VARV). Following intracellular metabolism of BCV to cidofovir-diphosphate (CDV-PP), CDV is incorporated by the viral polymerase into nascent chain virus DNA, inhibiting viral replication. Generation of resistance in orthopox viruses in vitro is typically slow (months), requires multiple mutations for high level resistance and resistant strains have impaired growth, suggesting a high barrier to resistance for BCV. BCV is available in tablet and liquid formulations, with an IV formulation in development; manufacturing has been validated at commercial scale. BCV has been administered to more than 1000 patients in our CMV, AdV, open label and named patient/emergency IND programs, including immune compromised patients, those with renal and/or hepatic impairment, and pediatric subjects as young as 1 month of age. The US FDA has agreed that the intradermal rabbitpox model and the intranasal mouse ectromelia virus (ECTV) model are well-characterized animal models of efficacy to support development of BCV for treatment of smallpox. In a pivotal rabbitpox efficacy study, animals received either placebo or BCV regimen consisting of an initial 20 mg/kg oral dose, followed by two 5 mg/kg doses at 48-hr intervals. BCV demonstrated 100% survival with treatment initiated at onset of fever, the first clinical sign of confirmed infection. Compared to the placebo mortality rate of 53%, a statistically significant reduction in mortality was observed when BCV was administered immediately, or at 24 or 48 hours after the onset of fever ($p < 0.05$ vs placebo, Fisher's exact test). A numerical reduction in mortality was also observed when BCV was initiated 72 hours after onset of fever, but this was not significant ($p = 0.091$ vs placebo). Further, BCV treatment was associated with a reduction in circulating infectious virus in infected rabbits, with earlier BCV intervention associated with a more profound reduction. Chimerix is conducting final studies in the ECTV mouse model and expects to complete the pivotal efficacy study with BCV in 2017. For the likely duration (3 weeks) and dose (200 mg/week) that will be recommended for BCV use in smallpox, BCV has demonstrated acceptable safety and tolerability in healthy subjects and in immunocompromised patient populations for whom a live vaccine is contraindicated.

This work was supported by a grant from NIH (1U01-A1057233-01) and an ongoing contract with BARDA (HHSO100201100013C).

FDA PERSPECTIVE ON THE DEVELOPMENT AND APPROVAL OF SMALLPOX MEDICAL COUNTERMEASURES

L. Borio

Acting Chief Scientist, 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. FDA also works to help foster the development of medical countermeasures—with the goal of achieving FDA approval⁹—as well as facilitating timely access to available 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 drugs, vaccines, and diagnostic tests. This presentation highlights the regulatory progress made in the development of smallpox medical countermeasures as well as some of the continuing challenges.

Smallpox medical countermeasures present unique and complex regulatory challenges because the scientific evidence upon which regulatory decisions are based is challenging to obtain owing to the fact that there is no smallpox disease in the world and animal models that adequately represent smallpox disease are not available.

FDA has worked closely with medical countermeasure developers to establish feasible and appropriate regulatory pathways for the approval of smallpox medical countermeasures. Since the last ACVVR meeting, FDA continues to see progress towards the development of medical countermeasures for smallpox, but there have also been some challenges. FDA's interactions with medical countermeasure developers has focused on providing feedback on proposed studies to support clinical safety, pharmacokinetic, and animal model efficacy studies for antiviral drugs, pivotal efficacy studies and bridging studies for the attenuated vaccines, and requirements for the validation of diagnostic tests.

⁹ The term “approval” refers to “FDA-approval, licensure, or clearance” under sections 505, 510(k), or 515 of the Federal Food, Drug, and Cosmetic Act or section 351 of the Public Health Service Act.

THE ROLE OF VACCINATION, ANTIORTHOPOXVIRUS DRUG, AND SOCIAL COOPERATIVITY IN A MATHEMATICAL MODEL OF SMALLPOX CONTROL

A. Kosaraju

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Considerable effort has gone into making mathematical and computer models of smallpox spread and control measures, typically consisting of vaccination and quarantine. Now that an antiorthopoxvirus drug is being stockpiled in the US, an outbreak model was constructed and published in the Journal of Biosecurity and Bioterrorism exploring antiviral use in combination with vaccination and social cooperativity to control an outbreak. A homogenously mixed, deterministic simulation of a single metropolitan area is considered. *Results:* Metropolitan-level mass vaccination coupled with drug treatment for all individuals who develop a fever considerably outperforms treating only those who develop smallpox's distinctive rash. More aggressive responses are more robust to low cooperation of the population with public health efforts and to faster disease spread. However, even with the most aggressive public health intervention, an attack that initially infects hundreds or thousands of individuals will need to be fought in multiple cities across the country.

Based on this model, an interactive visualization was developed in partnership with the McCain Institute in Washington DC. This visualization allows simultaneous toggling of a number of key outbreak parameters, such as the size of the initial attack, the speed of government interventions, cooperation of the population with vaccination, R nought and antiviral availability to determine the effect on three main outcomes: the mortality rates, the morbidity rates, and time to eliminate the outbreak from the population. After receiving an introduction to the incident scenario, the audience will have the opportunity to engage with the input variables to think through the best courses of action in an outbreak. This is the first published smallpox model to incorporate a smallpox antiviral and the first modeling visualization of its kind.

ANNEX 3. Agenda of the Eighteenth meeting of the Advisory Committee on Variola Virus Research

AGENDA

2-3 November 2016

Salle B, WHO Headquarters

Geneva, Switzerland

DAY ONE - 2 November 2016

- 9:00 – 9:15 OPENING – **Dr Peter Salama**, Executive Director, WHO Health Emergencies
- 9:15 – 9:30 Welcome remarks – **Dr Sylvie Briand**, Director, Department of Infectious Hazard Management, WHE
- 9:30 – 9:40 DA Henderson – In memoriam

VARIOLA VIRUS REPORTS

- 9:40 – 9:55 Report of the secretariat – WHO Smallpox Secretariat – **A. Khalakdina**
- 9:55 – 10:05 Update on Smallpox Vaccine Emergency Stockpile – **A. Costa**
- 10:05 – 10:15 Update on research proposals submitted to WHO in 2016 – **D. Evans**
- 10:15 – 10:45 **TEA/COFFEE BREAK**
- 10:45 – 10:55 Report on the variola virus collection at the WHO Collaborating Center Repository in VECTOR, Koltsovo, Novosibirsk Region, Russian Federation – **R. Shchelkunov**
- 10:55 – 11:05 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 – **I. Damon**
- 11:05 – 11:15 Update variola virus repositories biosafety inspection visits – **K. Kojima**

VARIOLA VIRUS RESEARCH UPDATE – 2016

ANIMAL MODELS

11:15 – 11:40 Use of live variola virus to determine whether mice are a suitable animal model for human smallpox (2015): update on disease pathogenesis – **C. Hutson**

DIAGNOSTICS

11:40 – 12:05 Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support– **I. Damon**

ANTIVIRALS

12:05 – 12:30 Use of live variola virus to evaluate antiviral agents against smallpox – **V. Olson**

12:30 – 13:30 **LUNCH**

VACCINES

13:30 – 13:55 Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of “third” generation vaccines – **V. Olson**

LICENSURE UPDATES

13:55 – 14:10 Update on LC16m8 vaccine – **H. Yokote**

14:10 – 14:25 Update on smallpox vaccine IMVANEX® (IMVAMUNE®) – **N. Samy**

14:25 – 14:40 Progress towards approval and deployment of Arestvyr® (ST-246) – **D. Hruby**

14:40 – 14:55 Update on the development of Brincidofovir (CMX001) for smallpox – **F. Gray**

14:55 – 15:10 FDA Perspective on the development and approval of smallpox medical countermeasures – **L. Borio**

15:15 – 15:45 **TEA/COFFEE BREAK**

PREPAREDNESS MEASURES

15:45 – 16:00 An interactive modelling approach to assessing the role of antivirals – **A. Kosaraju**

- 16:00 – 17:00 Discussion on criteria for approval of essential public health research –
D. Evans and Scientific Sub-Committee
- 17:00 – 17:30 GENERAL DISCUSSION

CLOSE OF DAY ONE

DAY TWO - 3 November 2016

Closed discussion for Members and Advisers of the ACVVR

- 09:00 – 10:30 Review of recommendations from 17th ACVVR on the implications of
synthetic biology on future variola virus research
- 10:30 – 11:00 **TEA/COFFEE BREAK**
- 11:00 – 12:30 Variola virus research priorities
- 12:30 – 13:30 **LUNCH**
- TEA/COFFEE (outside SALLE B)**

Closed final discussion for Members of the ACVVR

- 13:30 – 15:00 Decision on priority research questions
- Finalization of draft ACVVR report recommendations

CLOSE OF 18th ACVVR MEETING

ANNEX 4. List of participants

LIST OF PARTICIPANTS

Eighteenth meeting of the Advisory Committee on Variola Virus Research

2-3 November 2016

Salle B, WHO headquarters

Geneva, Switzerland

MEMBERS OF THE ADVISORY COMMITTEE

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OBSERVER

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Dr Sylvie Briand, Director, Department of Infectious Hazard Management, IHM/WHE

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Dr Catherine Makison Booth, Consultant, WHE

Mr Alejandro Costa, Team Lead, Vaccine Stockpiles, IHM/WHE

Dr Kazunobu Kojima, Global Capacities, Alert and Response

Ms Dhamari Naidoo, Technical Officer, HQ/EVO

Dr Susan Milner, Rapporteur

ANNEX 5. Terms of Reference of the Advisory Committee on Variola Virus Research

In 1980, the World Health Assembly declared the eradication of smallpox disease with the last case occurring in October 1977 in Somalia. Since then all remaining stocks of variola virus were moved to and are retained at two WHO global repositories, one in the Russian Federation and the other in the USA. The WHO Smallpox Secretariat, in WHO Health Emergencies programme, is responsible for ensuring the safe and secure management of these variola virus stocks by undertaking biennial biosafety inspections of the two repositories. In addition, the Secretariat also oversees all research activities on variola virus with the support of the Advisory Committee on Variola Virus Research (ACVVR).

The ACVVR was established in 1999 with expert members appointed by the Director-General upon the request of Health Assembly as set out in resolution WHA52.10. The group established what research, if any, must be carried out in order to reach global consensus on the timing for the destruction of existing variola virus stocks and, to develop a research plan for priority work on the virus. The ACVVR has met annually since then to continue their oversight on this research agenda. In May 2016, at the 69th World Health Assembly, Member States requested WHO to review the composition of the ACVVR to ensure that it had expertise in new biotechnologies and public health preparedness measures that would apply to a potential re-emergence of smallpox. This document outlines the work of the ACVVR which acts as an advisory body to WHO on matters of research-related strategy and variola virus stock management for the WHO Smallpox Secretariat.

Functions

In compliance with Resolution WHA52.10, which was adopted by the 52nd World Health Assembly on 24 May 1999; recalling that the group “will establish what research, if any, must be carried out in order to reach global consensus on the timing for the destruction of existing variola virus stocks” as per Resolution WHA52.10, the ACVVR shall have the following functions and make recommendations on these topics

1. advise WHO on all actions to be taken with respect to variola;
2. develop a research plan for priority work on the variola virus;
3. devise a mechanism for reporting of research results to the world health community; and
4. outline an inspection schedule to confirm the strict containment of existing stocks and to assure a safe and secure research environment for work on the variola virus.

Composition

1. The ACVVR shall have up to 25 members. The ACVVR members shall not be representatives of governments, organizations or institutions but rather shall serve in an independent, personal and individual capacity and shall represent the broad range of disciplines relevant to the work of the WHO Smallpox Secretariat. In the selection of the ACVVR members, primary consideration will be given to attaining an adequate technical distribution of expertise and schools of thought. Relevant technical areas include *inter alia* public health, virology, infectious diseases, diagnostics, vaccines, therapeutics, biosafety, biosecurity, biotechnologies, bioengineering, genomics and bioethics. Geographical representation and gender balance will be given due consideration in the selection process, to the maximum extent possible.

2. Members of the ACVVR, including the Chairman, shall be selected and appointed by WHO. For this purpose, the Director of the WHO Smallpox Secretariat, in consultation with relevant departments in WHO headquarters and regional offices, will propose a list of names based on the criteria mentioned above to the Executive Director of the WHO Health Emergencies Programme, for his/her consideration.
3. The Chairman's responsibilities shall include the following:
 - to direct the debate of the meeting of the ACVVR; and
 - to liaise with the WHO Secretariat between meetings.
4. Members of the ACVVR, including the Chairman, shall be appointed to serve for an initial term of three years, and shall be eligible for reappointment. Membership in ACVVR and designation as Chairman may be terminated at any time by WHO if WHO's interest so requires or as otherwise specified in these TORs or letters of appointment of the ACVVR members.
5. Representatives from partner organizations in official relations with WHO, may be invited by WHO to participate in ACVVR meetings as observers. Upon invitation of the Chair, they may present relevant scientific information or views and policies of their organizations and contribute to the discussions in the ACVVR. They will not participate in the process of adopting the final decisions or recommendations of the ACVVR.
6. ACVVR members must respect the impartiality and independence required of WHO. In performing their work, they may not seek or accept instructions from any Government or from any authority external to the Organization, even if they are employed by such government or authority. They must be free of real, potential or apparent conflict of interest. To this end, proposed ACVVR members will be required to complete a Declaration of Interest (DOI) form and their appointment, as well as their term's renewal, will be subject to the evaluation of completed DOI forms by the WHO Smallpox Secretariat, determining that their participation would not give rise to a real, potential or apparent conflict of interest.

Operation

1. The ACVVR shall usually meet face-to-face at least once each year. WHO shall provide any necessary scientific, technical and practical support for the ACVVR. WHO may convene additional meetings, including through teleconferences and videoconferences, on an ad hoc basis, as decided by the Executive Director of the WHO Health Emergencies Programme.
2. ACVVR members are expected to attend meetings. If a member fails to attend two consecutive ACVVR meetings, WHO may terminate his/her membership in the ACVVR. WHO may decide to appoint a member in replacement of that member.
3. Members are required to apprise themselves of the current state of knowledge about variola virus, and the arguments concerning the desirability of conducting further research on it. In order to assist the group of experts to obtain an overview on these topics, a series of formal presentations and discussions will be organized by the WHO Secretariat. It will be the duty of the Chairman to keep presentations and discussion to the time allotted in the agenda, and to moderate the discussion. In the

light of the information presented and the relevant discussion, the Members of ACVVR will make recommendations to the Director-General of WHO on issues related to whether or not further research on variola virus is required. If the committee reaches a decision by consensus, this decision will be recorded by the rapporteur, along with a summary of the supporting reasons. Should the committee be unable to reach a consensus, the rapporteur will record the majority and dissenting views, again with a summary of the supporting reasons.

4. The Members of the ACVVR will elaborate a research plan for essential public health research on variola virus. The procedures for organising and monitoring the necessary work and communication of results will be supported by a scientific sub-committee, which will be drawn from among the Members of the ACVVR. The principal role of this scientific sub-committee will be to help the WHO Secretariat in facilitating the approval and scientific oversight of variola virus research activities. The subcommittee may include additional specialists selected to complete the scientific expertise required.

5. Reports of each meeting will be submitted by the ACVVR to WHO (the Executive Director of the WHO Health Emergencies Programme). All recommendations from the ACVVR are advisory to WHO, who retains full control over any subsequent decisions or actions regarding any proposals, policy issues or other matters considered by the ACVVR. WHO also retains full control over the publication of the reports of the ACVVR, including whether or not to publish them.

6. Information and documentation to which ACVVR members may be given access in performing ACVVR related activities will be considered as confidential and proprietary to WHO and/or parties collaborating with WHO. ACVVR members shall not purport to speak on behalf of, or represent, the ACVVR or WHO to any third party. Prior to confirmation by WHO of their appointment as ACVVR members, ACVVR nominees will be required to sign a WHO confidentiality undertaking and provisions on ownership.