



WHO Advisory Committee on Variola Virus Research

Report of the Sixteenth Meeting

Geneva, Switzerland

20 and 21 October 2014



World Health
Organization

PANDEMIC AND
EPIDEMIC DISEASES

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Printed by the WHO Document Production Services, Geneva, Switzerland.

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*WHO Advisory Committee on Variola Virus Research
Report of the Sixteenth Meeting, Geneva, Switzerland, 20 and 21 October 2014*

Executive summary

On 20 and 21 October 2014 the Advisory Committee on Variola Virus Research held its sixteenth meeting.

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 Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America (USA).

All the proposals for research in 2013-2014 that involved live variola virus and that had been approved by the scientific subcommittee were requests for extensions of existing approved proposals. No new proposal for research involving live variola virus was approved (one was received but not approved).

The Advisory Committee also received updates on the use of live variola virus for the refinement of diagnostic tests, the search for an animal model of smallpox, antiviral studies, and the status of vaccine development.

In the USA, a DNA-based diagnostic test for variola virus has been refined and is entering final stages of validation. In the Russian Federation, documentation has been prepared and the necessary arrangements made for government licensing of a DNA-based assay for species-specific diagnosis of variola virus and other pathogenic orthopoxviruses.

Participants from CDC reported the discovery of vials labelled as variola virus found in a laboratory in the USA. These vials were transported under high security to the CDC laboratories under the existing programme at the WHO Collaborating Centre. The vials were found to contain viable variola virus by real-time PCR tests and underwent full genomic sequencing. These are now held in the secure CDC facility awaiting destruction. A thorough search of all government laboratories in the USA was initiated and revealed no further variola virus material.

The discovery of smallpox material on display in the National Museum of Prague was also reported. A small portion of this material was extracted and sent under high security to a European laboratory for testing; molecular assays and sequencing confirmed that it contained smallpox material. Nevertheless, DNA analysis indicated that the variola genomes were considerably degraded and therefore it was unlikely that the material contained viable virus.

Two antiviral compounds (tecovirimat, ST-246, and brincidofovir, CMX-001) that are in the process for registration in the USA do not require further animal model research with live variola virus for review by the Food and Drug Administration. Representatives of two pharmaceutical companies described progress toward the registration of these two candidate antiviral agents.

Considerable progress has been made towards the testing and registration of highly attenuated smallpox vaccines that would be suitable for use in people that are immunologically

compromised. Some of these vaccine stocks have been shown to be stable in a range of storage conditions.

The estimated smallpox vaccine stocks at global level are about 700 million doses (country and WHO stock).

1. Report of the WHO Secretariat

- 1.1 The WHO Advisory Committee on Variola Virus Research met on 20 and 21 October 2014 under the chairmanship of Professor G. Smith, with Dr A. Robinson as Rapporteur.
- 1.2 Dr S. Briand opened the meeting and reviewed the activities that had taken place since the last meeting of the Advisory Committee in September 2013.¹ The Advisory Committee had recommended that no further research was needed at the two collaborating centres that required live variola virus for vaccine development and diagnostics. The majority of the members of the Advisory Committee saw the necessity to retain live variola viruses for further work on antiviral agents. The Advisory Group of Independent Experts to review the smallpox research programme (AGIES) had also been asked to consider whether live virus needed to be retained.² It considered that there was no need for the virus to be retained. The recommendations of the two committees were noted by the Sixty-seventh World Health Assembly in May 2014, which also agreed to the establishment of a group of experts to advise the Director-General on the implications of synthetic biology and new technologies.³

Dr Briand raised the important issue of the Ebola outbreak and how the work of WHO on smallpox has assisted the response to Ebola in terms of deployment of vaccines, legal aspects and the development of antiviral agents.

- 1.3 Dr A. Costa reported on the work of the Secretariat. The cloned DNA material in the National Institute for Communicable Diseases in South Africa was destroyed in January 2014, following an adapted protocol based on recommendations from the Ad Hoc Committee on Orthopoxvirus Infections. Six samples of variola virus had been found at the National Institutes of Health in the USA and transported to CDC. WHO had been informed of the genetic sequencing that had been conducted. The samples are awaiting destruction, which is planned for the beginning of 2015.

WHO had organized a consultation of the Strategic Advisory Group of Experts on immunization in 2013 to review the safety/efficacy data on smallpox vaccines and advise on which vaccine should be recommended for use in a smallpox outbreak. The Group comprised a mixture of experts on smallpox, virology, immunology, vaccinology, public health, ethics and regulatory matters who provided an independent review of all clinical data on efficacy, safety and effectiveness and advice on vaccine recommendations, composition and size of the WHO smallpox vaccine stockpile. WHO started to develop emergency prequalification mechanisms to ensure

¹ WHO Advisory Committee on Variola Virus Research: Report of the Fifteenth Meeting, document WHO/SE/PED/CED/2013.2 (http://apps.who.int/iris/bitstream/10665/97033/1/WHO_HSE_PED_CED_2013.2_eng.pdf, accessed 20 December 2014).

² Advisory Group of Independent Experts to review the smallpox research programme (AGIES): report to the World Health Organization, document WHO/HSE/PED/CED/2013.3 (http://www.who.int/csr/resources/publications/PED_SmallpoxAGIES_report2013.pdf, accessed 20 December 2014).

³ See summary records of the twelfth meeting of Committee A of the Sixty-seventh World Health Assembly, section 8 (http://apps.who.int/gb/ebwha/pdf_files/WHA67-A-B-PSR/A67_ASR12-en.pdf, accessed 20 December 2014).

timely deployment in countries of smallpox vaccines in case of an outbreak. WHO has accepted a vaccine donation from the USA, and the Secretariat is negotiating with the governments of France and Japan about vaccine donations. The operational framework for vaccine deployment has been finalized. The stock currently consists of 35 million doses.

The Advisory Committee was also informed of the request made by the World Health Assembly for the Director-General to organize a consultative process on the implications of the use of synthetic biology technology on smallpox preparedness and response.

The biosafety inspection of the repository at VECTOR is planned for December 2014, and the comparable inspection at CDC is planned for May 2015.

The current coordinator of the scientific subcommittee has indicated that he no longer wishes to coordinate the review of the research proposals. The Secretariat will review the membership of the subcommittee and appoint a new coordinator.

2. Update on WHO-approved research proposals

- 2.1 Dr R. Drillien reported that eight proposals submitted to the Advisory Committee's scientific subcommittee in 2013-2014 had been recommended for approval by a majority of the subcommittee's members (see Annex 1¹).

3. Update on variola virus stocks held in the repositories in the Russian Federation and the United States of America

- 3.1 Professor A. Agafonov reported on the status of the collection of strains and materials held in the variola virus repository at VECTOR, Koltsovo, Novosibirsk Region, Russian Federation. Organization of and experimentation with materials in the variola virus collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at VECTOR are in compliance with national and international biosafety and biosecurity requirements and the recommendations of the WHO Advisory Committee on Variola Virus Research and those of the WHO biosafety and biosecurity inspection missions.

Currently, the variola virus collection comprises 120 strains, originating from countries in Africa, South America, Asia, Europe and the Eastern Mediterranean. The collection contains freeze-dried and frozen cultures (120 strains) and 17 primary specimens isolated from human patients in the past. The total number of registered stored units is 692.

¹ Abstracts of all the presentations are contained in this Annex.

In 2014, the Ind-3a variola virus strain was grown in Vero cells and used to assess in cell culture the antiviral properties of synthetic compounds and the neutralizing activity of sera from vaccinated individuals and immunized laboratory animals. Four vials of Ind-3a were removed from the working stocks and, upon completion of the research, all the remaining working aliquots were destroyed.

Proposals for research using live variola virus for 2015 will be submitted to WHO for approval and involve work:

- to assess the potential of new antiviral agents for smallpox treatment and prevention
- to measure variola virus neutralizing activity of sera from people vaccinated against smallpox
- to develop animal models to study the efficacy of therapeutic and preventive products against smallpox.

3.2 Dr V. Olson reported on the status of the collection at the CDC (Atlanta, Georgia, USA). The WHO Collaborating Centre for Poxviruses in CDC continues to maintain one of the two consolidated, international collections of variola virus strains. The laboratory space was in active use from October 2013 until late March 2014. The laboratory then underwent decontamination before preventative maintenance in April 2014. The laboratory became operational again in early June 2014. No new variola virus seed pools were added to the inventory between 2013 and 2014. Working stocks of two crude variola virus strains have been prepared for use in evaluating protein-based diagnostics and next generation vaccines. The six vials of variola virus found on the Bethesda campus of the National Institutes of Health in July 2014 have been inventoried and temporarily stored in a secure freezer awaiting destruction to be witnessed by a WHO representative. WHO-approved research activities that have used variola virus, or products from prior studies using variola virus, from the inventory within the past year have focused on verification of viable virus in animal samples (either swabs or necropsy material), testing in tissue culture of promising antivariola agents, investigation of the host kinome response to look for potential therapeutic targets, optimization of protein-based diagnostic assays, and neutralization efficacy studies of sera obtained through use of next-generation vaccination regimens

In May 2013, historical samples from cases diagnosed as smallpox were removed from the repository for viral sequencing, according to a WHO-sanctioned protocol with no viral propagation. Whole genomic libraries were prepared at the WHO Collaborating Centre before sequencing. The original homogenates from chicken egg chorioallantoic membranes proved problematic for full genomic sequencing. Procedures for genomic library construction have been optimized and sequencing efficiency has been greatly improved. To date, 135 original samples have been processed with 42 samples having undergone complete genomic sequencing. The sequence data are awaiting assembly and annotation.

4. Update on laboratory diagnostic tests

- 4.1 Dr Olson also presented an update on the use of live variola virus for the development of diagnostic tests for variola virus based on nucleic acid or protein detection systems. The ability to validate nucleic acid-based and protein-based diagnostic capacity is crucial for early detection and recognition of smallpox should it re-emerge. The consequences of either false-negative or false-positive results would be significant for the global public health system.

DNA diagnostics

Following the finding in 2010 of cross-reactivity of several strains of cowpox virus with a previously-validated sequence considered to be unique to variola virus, a new set of variola virus signature sequences has been identified and characterized for specificity and sensitivity to variola virus detection assays. Such assays have been refined and, through work with the United States Laboratory Response Network Technical Review Committee and FDA, are entering final stages of validation, having completed a multicentre reproducibility study with a plasmid containing non-contiguous assay target fragments of variola virus nucleic acid.

Protein diagnostics

The ability of a rapid, column-based orthopoxvirus diagnostic assay developed by CDC's collaborators at the Robert Koch Institute in Berlin to detect variola virus was validated. The diagnostic assay has similar sensitivity for variola virus as for vaccinia virus and provides a result in less than one hour. The assay is easy to use and does not rely on any expensive equipment. Since late 2011, studies have also been continuing on monoclonal antibody characterization and viral antigen-capture assays. This work has focused on standardizing virus preparations, re-subcloning promising hybridomas, and bioinformatics-guided approaches to variola-specific monoclonal antibody design. Among peptide-derived monoclonal antibodies that have been screened for reactivity against gamma-irradiated variola virus samples and whose specificity has been compared with that for other orthopoxviruses, several have shown promise. New diagnostic assays using these antibodies may allow for rapid detection and speciation of variola virus with antigen capture assays.

- 4.2 Professor S. Shchelkunov described a multiplex real-time PCR (MuRT-PCR) assay for orthopoxviral DNA, in which four species-specific pairs of oligonucleotide primers and four species-specific hybridization tests were used simultaneously with various fluorescent dyes and their respective quencher agents. Respective dye/quencher pairs were specific for variola, monkeypox, cowpox and vaccinia viruses. The specificity and sensitivity of the method were assessed by analysis of DNAs of 29 strains of six species of these orthopoxvirus, as well as DNA samples isolated from historical clinical materials from human smallpox cases and experimental materials recovered from mice infected with cowpox virus and marmots infected with monkeypox virus. The assay is more specific and faster than an earlier multiplex PCR method for species-specific gene identification of human pathogenic orthopoxviruses.

During 2014, documentation was prepared and the necessary arrangements made for government licensing of the MuRT-PCR assay for species-specific diagnosis of variola virus and other pathogenic orthopoxviruses.

- 4.3 Dr L. Golightly described the development of multiplexed detection assays that use species-specific PCR followed by ligase detection reactions. The resultant fluorescently-labelled ligation products have been detected on a universal array or bead express instrument, enabling the simultaneous detection and identification of multiple pathogens. The assay has been evaluated on 53 different isolates of viruses associated with haemorrhagic fevers (Ebola virus, Marburg virus, Crimean-Congo hemorrhagic fever virus, Lassa fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus) as well as variola virus. The assay detected all viruses tested including eight sequences representative of different variola virus strains from the CDC repository. No cross-reaction was observed with other viruses of emerging zoonoses such as monkeypox virus or cowpox virus, or six flaviviruses tested (St. Louis encephalitis virus, Murray Valley encephalitis virus, Powassan virus, tick-borne encephalitis virus, West Nile virus and Japanese encephalitis virus). These multiplexed assays are poised for development in a high-throughput real-time format to provide accurate determination of viral load and viral identification in urban settings. In addition, point-of-care diagnostics (e.g. powered by cell phones) are being developed.

5. Update on animal models

- 5.1 Dr D. Carroll reported progress on evaluation of the CAST/EiJ mouse model for variola virus infection. The discovery of a novel, permissive/representative animal model system would facilitate the development of new, safer smallpox vaccines and therapeutics. Recent investigations have shown that CAST/EiJ mice are highly susceptible to a range of orthopoxviruses. However, although some variola virus replication was detected and some illness was observed, the CAST/EiJ mouse model is not suitable for further smallpox vaccine and therapeutic studies.
- 5.2 Dr A. Agafonov reported on studies to assess the possibility of using ICR and inbred immunodeficient SCID mice infected intranasally with variola virus (Ind-3a strain) as a laboratory model of smallpox.

The patterns of variola virus accumulation in the organs and tissues of ICR mice following intranasal inoculation with variola virus were studied. The virus was detected in the brain, nasal septum and lungs only, indicating the absence or very low viral sensitivity of the cells in other organs. For SCID mice, a similar pattern was observed, but in contrast to ICR mice, no virus was detected in the brain but was found in the trachea; the highest titres of variola virus were found in the lungs. The level of virus found in the lungs of these mice made it feasible to use them to assess the activity of anti-smallpox therapeutic and prophylactic products. One synthetic compound (NIOCH-14), which had been shown to be effective against monkeypox virus infection in both ICR and SCID mice, was tested in ICR mice infected

intranasally with variola virus. Virus titres in the lungs of mice treated with NIOCH-14 were significantly lower than in untreated mice and no virus was detected in the lungs of four mice. It was concluded that the ICR mouse/variola virus model would be a useful model to assess the efficacy of antivariola virus agents.

6. Update on vaccines

- 6.1 Dr Olson reported results of collaboration between CDC, the National Institute of Infectious Diseases in Japan and pharmaceutical industry scientists on the continuing evaluation of vaccination regimens for new smallpox vaccines, specifically LC16m8. The development of new vaccines has focused on the use of attenuated vaccine strains, such as Modified Vaccinia Ankara (MVA) and LC16m8. These vaccines, however, were not tested directly for efficacy against smallpox during the eradication campaign as most were developed towards the end of that era. The ability of sera, generated through trials with these less reactogenic smallpox vaccines, to neutralize variola virus may provide a surrogate indicator of protection in vaccinees. Variola virus neutralization as a marker for vaccine efficacy is particularly valuable for the evaluation of vaccines (such as MVA) that do not elicit a “take”, the traditional measure of vaccine success.

The collaborative studies showed that vaccination with LC16m8 induced a neutralizing immune response that was not significantly different from that following vaccination with Dryvax. Vaccinees’ sera were also evaluated for neutralizing capacity against different strains of variola virus. Although Dryvax vaccinee sera did not show a significant difference in ability to neutralize the two strains of variola virus, LC16m8 vaccinees’ sera demonstrated greater neutralization ability against the strain from primary clade II. Although the numbers of samples evaluated were small (4 subjects were vaccinated with Dryvax and 10 with LC16m8), the same significant difference in neutralization ability was observed when sera from 10 more LC16m8 vaccinees were evaluated. Continued study of vaccinees’ sera will help to define differences that may exist between neutralization ability for different strains of variola virus. Progress has also been made in designing assays to determine neutralizing capacity of MVA vaccinees’ sera against variola virus. These data will be essential for future submissions towards regulatory approval for these third-generation vaccines.

- 6.2 Dr Shchelkunov reported that, in the course of Phase 2 clinical trials of the tablet-formulated live recombinant egg-based smallpox vaccine Revax-BT, the titres of antibodies against variola virus in the sera of vaccinated volunteers were assessed 6 and 12 months after vaccination. The levels of specific antibodies in the sera were determined by neutralization of the Ind-3a strain of variola virus. Vaccination with Revax-BT induced variola virus neutralizing antibodies in all vaccinated volunteers and the titres of the antibodies in the blood of the volunteers are maintained for at least a year.

He also described the ability of novel investigational smallpox vaccines to induce, in animals, levels of antibodies capable of neutralizing variola virus comparable to those

induced by conventional smallpox vaccine. In guineapigs immunized with one dose of cell-based microencapsulated recombinant smallpox vaccine, high levels of antibodies neutralizing the Ind-3a strain of variola virus were observed after 30 days in all the animals.

Studies were conducted in mice of the immunological efficacy of a genetically modified cell-based smallpox vaccine (GMCS vaccine), derived from a modified LIVP vaccinia virus strain. This attenuated vaccine was compared with that based on the unmodified LIVP vaccinia virus strain. Serum samples from the mice were tested at 28-30 days post immunization. A single subcutaneous immunization of mice with the GMCS vaccine in a dose of 5.0-5.5 log PFU resulted in the formation of antibody levels neutralizing the Ind-3a strain of variola virus in 100% of the animals. This was the same level of neutralization as seen after subcutaneous immunization with the LIVP vaccine at the same dose.

- 6.3 Dr N. Arndtz-Wiedermann reported on progress with registration of the MVA-based smallpox vaccine Imvanex® (Imvamune® in the USA and Canada). In 2013, the European Medicines Agency registered the vaccine (as Imvanex®), the authorization covering active immunization against smallpox for the general adult population, including people with weakened immune systems. Later that year, Health Canada registered the vaccine for persons of 18 years of age and older who have contraindications to traditional replicating vaccines. In the USA the vaccine is under regulatory evaluation by FDA.

The vaccine has been tested in more than 7500 subjects including 1000 with contraindications for replicating smallpox vaccines, including HIV infection and atopic dermatitis. In 20 completed or ongoing clinical trials it has demonstrated a favourable safety profile in both healthy individuals and people with impaired immune function and active atopic dermatitis.

In November 2013 WHO accepted a donation of the vaccine from the USA for inclusion in its stockpile. The vaccine also currently constitutes part of the United States Strategic National Stockpile of medical products to which a further four million doses are currently being delivered to supplement the 24 million doses already delivered. In August 2014 both the Canadian health and military authorities upgraded their preparedness to include the vaccine.

Current stockpiled replicating vaccines do not allow for vaccination of all individuals; between 5% and 25% of a given population are not eligible. The approval of this non-replicating vaccine facilitates immunization of first-line responders and military personnel in preventive settings, as replicating vaccines are associated with a high rate of severe side effects. Approval of the vaccine provides an opportunity to update preparedness plans and immunization strategies.

- 6.4 Dr H. Yokote reported on stability studies on LC16m8 vaccine, a lyophilized product. Its current authorized shelf-life is four years when stored at -20 °C, but results of an ongoing study suggest a longer shelf-life. In addition, five-year stability data have been obtained for vaccine bulk materials. The studies confirm the high stability of the

vaccine in a range of storage conditions. The research was driven by the need to respond to demand for large amounts of the vaccine. Typically, it takes six to nine months from an order to delivery of the vaccine, when the vaccine is manufactured de novo from raw materials, but when the vaccine is manufactured from the vaccine bulk materials, the time to delivery can be shortened to three to four months. The stock of the bulk materials will be sufficient to meet demand from overseas within a satisfactory period.

7. Update on antiviral agents and therapeutics

- 7.1 Professor Shchelkunov reported that in 2013-2014 more than 70 novel chemically synthesized compounds belonging to three different series were tested in vitro at VECTOR for their ability to inhibit the replication of various surrogates of variola virus. The antiviral activity of 19 compounds, including 15 of the NIOCH series of pyrrolidinediones and derivatives, was evaluated in cell culture against variola virus strain Ind-3a. Four pyrrolidinedione complexes showed good activity and are considered promising candidates for further in vivo studies. However, their activity was lower than that of tecovirimat.
- 7.2 Dr Olson reported on the use of live variola virus to evaluate antiviral agents against smallpox in research approved by the Advisory Committee's scientific subcommittee. Considerable progress has been achieved in developing tecovirimat and brincidofovir (the orally available derivative of cidofovir. Research with multiple strains of variola virus containing various non-synonymous coding changes in the viral drug target (the viral DNA polymerase) established that brincidofovir was about 100 times more potent than cidofovir.

A recently described pyridopyrimidinone inhibits replication of vaccinia virus, cowpox virus and monkeypox virus. It appears to inhibit the poxviral DNA-dependent RNA polymerase, and in vitro assays show that it limits RNA synthesis of intermediate and late viral genes (as is seen with vaccinia virus). A transcription inhibitor may be of significant therapeutic benefit by stopping expression of poxvirus immunomodulatory proteins. Members from both clades of variola virus are sensitive to inhibition by pyridopyrimidinones.

The complete kinome analysis identified potential host kinases as therapeutic targets. Identification of such novel targets may allow "repurposing" of medicines, currently approved by FDA for other indications, in the treatment of orthopoxvirus infections. Preliminary evaluation has identified two compounds (sorafenib and crizotinib) that limit variola virus multiplication, but the stage of the viral life cycle at which these compounds function is not known.

- 7.3 Dr D. Hruby presented an update on progress towards regulatory approval of tecovirimat. With the evolution of the "animal rule" in the USA the company manufacturing tecovirimat has reacted in order to provide the necessary data to

demonstrate both efficacy and dose justification. In addition to previous animal experimentation, notably studies of monkeypox in non-human primates, the company is now working on rabbitpox in rabbits to provide a robust set of data in a second species to support the non-human primate studies. Preliminary experiments demonstrate that tecovirimat can still demonstrate therapeutic benefit when given to infected rabbits as late as five days post infection. Dr Hruby also provided an update on the delivery of tecovirimat to the US Strategic National Stockpile. At present, delivery of the entire two million course order is expected to be completed in the second quarter of 2015.

- 7.4 Dr L. Trost described the corporate structure of the company developing brincidofovir and the nature of its relationship with the Biomedical Advanced Research and Development Authority in the USA (BARDA) to develop the drug as a treatment of smallpox. The drug inhibits replication of many viruses including adenovirus, cytomegalovirus and variola virus. It has been administered to more than 1000 human subjects, many with life-threatening viral illnesses and including children as young as one month of age. Safety and efficacy data from clinical development of brincidofovir for other indications have informed the smallpox development programme and a current Phase 3 trial for prevention of cytomegalovirus disease in immunocompromised transplant patients is designed with doses that are also proposed for treatment of smallpox. The FDA and the company developing brincidofovir have agreed to the design of an efficacy study to be initiated in the intradermal rabbitpox model in rabbits in late 2014. In August 2014, BARDA announced a US\$ 17 million extension of its contract with the company to continue to develop brincidofovir for treatment of smallpox. The results of recent collaborative studies show that treatment with brincidofovir does not impede development of protective immunity in animals recovering from vaccinia virus infection nor the successful vaccination with either replicating (Dryvax, ACAM2000) or non-replicating (MVA) smallpox vaccines. If approved, brincidofovir would be available in tablet and liquid formulations.

8. Regulatory issues

- 8.1 Dr L. Borio described FDA's regulatory progress, in particular its work on medical countermeasures for smallpox, whose development it fosters, with the goal of achieving FDA approval, as well as facilitating timely access to them in the event of a public health emergency. The US government is supporting such work, including the development of antiviral agents, vaccines and diagnostic tests. Regulatory mechanisms for access to medical countermeasures during public health emergencies are based on benefit/risk assessments anchored in scientific evidence. As medical countermeasures against smallpox present unique and complex regulatory challenges, FDA is working closely with their developers, aiming to establish feasible and appropriate regulatory pathways for approval. It provides feedback on proposed studies to support safety, pharmacokinetic and animal model efficacy studies for antiviral agents and efficacy studies for the attenuated vaccine.

9. DNA vials at the National Institutes of Health in the United States of America

- 9.1 Dr D. Carroll reminded the meeting that, before the smallpox eradication campaign, variola virus was routinely studied in numerous laboratories. Forgotten glass ampoules storing some of these historic samples were found in a refrigerated space at the Bethesda campus of the National Institutes of Health some 60 years after initial storage. The samples were carefully inventoried by CDC staff and all unknown samples or those designated as variola virus were transferred to the CDC's biosafety level 4 laboratory for processing and characterization. Real-time PCR confirmed the presence of variola virus DNA in the six vials labelled "variola virus" or "alastim". These samples contained viable virus and underwent full genomic sequencing. The virus isolates generated four sequences that matched previously sequenced isolates and two unique sequences. The new sequences expand our knowledge on the diversity of variola virus strains. The samples are currently held securely at CDC awaiting destruction to be witnessed by a WHO representative.

A subsequent thorough search of government laboratories in the USA revealed no further variola virus material.

10. Update on biosafety inspections of the variola virus repositories

- 10.1 Dr K. Kojima described the preparations for the planned biosafety inspections of the two authorized repositories of variola virus (CDC and VECTOR) due to be undertaken in 2014-2015. The last inspections were done in 2012 and the planned inspections fall in the two-year cycle specified in resolution WHA60.1. Discussions with the authorities in the two repositories have resulted in informal concurrence that the onsite inspections take place for VECTOR in December 2014 and CDC in May 2015. The timing of the visits is synchronized with the period when each facility in question will be "cold" or decontaminated for scheduled maintenance.

A group of experts with different backgrounds has been identified to provide the expertise necessary for the inspections in a team led by WHO. In the inspections in 2012, the WHO's protocol was based on the European Committee for Standardization (CEN) Workshop Agreement CWA 15793:2011. The experts for the planned inspections met recently to review this approach and to adapt it to the relevant needs of the biorisk management assessment of the two centres.

11. Synthetic biology technology for smallpox preparedness and response

- 11.1 The Secretariat presented a draft proposal for the establishment of an independent WHO committee to examine the implications of use of biological synthetic technology on smallpox preparedness and response.

The discussion that followed raised questions about the need for another committee. Considering the question of whether an infectious variola virus could be recreated by synthetic biology, members of the Advisory Committee recommended the following:

(a) Infectious orthopoxviruses (different strains of vaccinia virus and cowpox virus) have been derived from cloned DNA and these results have been reported in scientific journals by several groups in different countries. Although these viruses were not created from synthetic oligonucleotides, as was done for instance with poliovirus, there is no logical impediment to this synthetic route being possible. Indeed, the increasing ease and reduced cost of constructing long DNA strands makes that synthetic process less complex.

(b) The creation of live variola virus by any method, including rescue of virus in cell culture from DNA, is prohibited absolutely. However, the technology associated with the recreation of vaccinia virus and cowpox virus is known and could be applied to variola virus. There is no reasonable doubt that recreation of live variola virus from either synthetic oligonucleotides or cloned DNA is possible.

Given the above, the Advisory Committee considered that the creation of a committee to discuss this issue was unnecessary because the answer is already known. They also considered that all matters concerning live variola virus are the remit of the Advisory Committee; that another committee was unnecessary; and that multiples lines of reporting on the same issue might not be helpful.

This approach would avoid fragmentation of expertise present in existing committees addressing the question of synthetic biology as related to variola virus. The Advisory Committee recommended that issues concerning the recreation of variola virus by synthetic biology should remain within its remit but that the larger issue of the recreation of dangerous pathogens by synthetic biology in general and its implications for regulatory and policy issues would be a useful topic for WHO to evaluate.

12. Other presentations, including historical samples at the National Museum of Prague

- 12.1 Professor G. Smith gave a presentation on behalf of Dr H. Meyer, Head of BSL-3 Laboratory, Bundeswehr Institute of Microbiology, München, Germany, on the discovery of historical samples of human smallpox lesions on display at the National Museum of Prague. These samples consisted of parts of limbs and skin that showed lesions indistinguishable from clinical smallpox. Sequencing of material extracted

from these lesions identified two fragments of variola virus DNA, although the DNA fragment size was small. One DNA sequence was found to be closely related to sequences in the variola virus DNA database of a virus derived from the Indian subcontinent, and the second was unique and more distantly related to sequences of the West-African/alastrim clade.

13. General discussion

13.1 The Chairman summarized the work presented at the meeting.

The Advisory Committee had reviewed the work using live variola virus that had been undertaken during the past year in the two collaborating centres. It noted that there had been a limited amount of work using live variola virus at the two centres and that there has been no reduction in the total number of samples of live virus retained.

Vaccines

Work reported on vaccine development included stability of vaccine stocks, ongoing trials to evaluate vaccine efficacy by non-inferiority studies in comparison to licenced smallpox vaccines, and plaque reduction neutralization studies with live variola virus.

Diagnostics

Further refinement was reported of existing PCR-based detection systems that had hitherto been considered variola virus-specific, to ensure that they identify viral DNA and distinguish the virus from other orthopoxviruses. This work was driven by the discovery of a cowpox virus isolates containing regions of DNA identical or closely similar to variola virus. This lack of specificity was overcome through the design of diagnostic assays targeting different regions of the variola virus genome. These new assays were tested on previously unsequenced variola virus samples.

Protein-based detection systems using monoclonal antibodies that recognized variola virus antigens with some degree of specificity were reported. A portable column orthopoxvirus generic protein-based diagnostic assay was able to detect a low level of variola virus within 45 minutes. Protein detection systems reported so far are not as sensitive as DNA detection systems.

Antiviral agents

Progress toward registration of the two leading compounds, tecovirimat and brincidofovir, was reported. The FDA animal efficacy rule for smallpox has undergone some evolution and a “triangulation” model has now been deemed acceptable for these two compounds. This move has been necessary, as an animal/variola virus model that closely mimics smallpox in human beings has not been found. As a result, the efficacy of these two compounds in animals infected with orthopoxviruses other than variola virus can be used as surrogate evidence of efficacy against variola virus in humans. Work with tecovirimat in a monkeypox

virus/non-human primate model and rabbitpox virus in rabbits and similar work with brincidofovir against ectromelia virus in mice and rabbitpox virus in rabbits are progressing well. Further work was ongoing, but it was noted that this was not scheduled to include work with live variola virus.

Work on a broad range of other antiviral compounds at both collaborating centres is in earlier stages of development as therapeutics for smallpox. The Advisory Committee considered that such work should be carefully monitored by the Committee to ensure that it was performed only with compounds that had a reasonable chance of success as an antiviral agent and that the precise type of research done was essential for public health benefit.

Annex 1. Abstracts of presentations

**RESEARCH PROPOSALS SUBMITTED TO WHO IN 2013-2014 AND
RECOMMENDED FOR APPROVAL BY THE SCIENTIFIC SUBCOMMITTEE**

Scientific subcommittee members: Clarissa Damaso, Grant McFadden, Andreas Nitsche, Jean-Claude Piffaretti, Tony Robinson, Li Ruan, Oyewale Tomori, Robert Drillien (coordinator)

Project proposals from CDC

27 November 2013 (review date)

Use of live variola virus to evaluate antivirals against variola

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

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

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

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

7 May 2014 (review date)

Use of live variola virus in systems kinomics for identification of host targets for therapeutic purposes

Project proposals from VECTOR

14 January 2014 (review date)

Development of animal models to study the efficacy of therapeutic and preventive products against smallpox

Discovery of new antivirals for smallpox treatment and prevention

Assessment of the neutralizing activity of vaccine blood sera using live variola virus

REPORT ON THE VARIOLA VIRUS COLLECTION AT THE WHO COLLABORATING CENTRE REPOSITORY IN SRC VB VECTOR

Dr Alexandr Agafonov

FBRI SRC VB VECTOR, Koltsovo, Novosibirsk Region, Russian Federation

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 SRC VB VECTOR is in compliance with national and international requirements, and the recommendations of the WHO Global Commission. Instructions regulating research, as well as all maintenance and control procedures, have been developed on the basis of the documents listed above. Plans have been developed for anti-epidemic measures and response to accidents. Emergency teams have been established for activation in case of accidents or emergencies.

Currently, the VARV collection comprises 120 strains, originating from countries in Africa, South America, Asia, Europe and the Eastern Mediterranean. According to an inventory inspection, the Russian collection of variola virus strains contains:

- freeze-dried and frozen cultures: 120 strains;
- 17 primary specimens isolated from human patients in the past.

The total number of registered stored units: 692.

In 2014, the Ind-3a variola virus strain was grown in Vero cells and then it was used to assess:

- the antiviral properties of chemically synthesized compounds, in cell culture;
- the neutralizing activity of sera collected from vaccinated individuals and immunized laboratory animals, in cell culture; and
- the susceptibility of mice to VARV.

For this purpose, four vials of Ind-3a were removed from the working stocks and they were used to prepare the desired quantities of virus aliquots to be used during the entire research campaign. Upon completion of the research campaign, the remaining working aliquots were destroyed.

Research using live variola virus is going to be continued in 2014-2015 to conduct the following research activities:

- discover new antiviral chemically synthesized compounds for treatment and prevention of smallpox;
- assess variola virus neutralizing activity of sera from those vaccinated against smallpox;
- develop 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, GEORGIA: 2014 REPORT ON THE VARIOLA COLLECTION**

Victoria Olson, Paul Hudson, Ashley Kondas, Zachary Braden, Scott Smith, Christine Hughes, Inger Damon

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

The WHO Collaborating Center for Poxviruses in Atlanta, Georgia, 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 US Select Agent requirements have been constructed to track usage of variola virus. Annual reports on the status of these collections are provided to the WHO. No new variola virus seed pools were added to the inventory between 2013 and 2014. Working stocks of two crude variola virus strains (NIG69_001, and SLN68_258) have been prepared for use in evaluating protein-based diagnostics and next generation vaccines, respectively. The six vials of variola virus found on the National Institutes of Health Bethesda campus in July 2014 have been inventoried and temporarily stored in our secure freezer awaiting destruction, witnessed by a World Health Organization representative. WHO-approved research activities which have utilized variola virus, or products from prior studies using variola virus, from the inventory within the last year have focused on: sample analysis of previously performed animal studies, tissue culture analysis of promising compounds for anti-variola virus activity, evaluation of the variola virus elicited host kinome response to look for potential therapeutic targets, optimization of protein based diagnostic assays, and evaluation of sera from vaccination regimens to evaluate efficacy based on variola virus neutralization.

In May 2013, variola virus samples were removed from the repository for WHO-sanctioned protocols. These original scabs or homogenates were processed (not propagated) for sequencing under the WHO-approved protocol. The laboratory space was in active use from October 2013 through late March 2014; the laboratory underwent decontamination prior to preventative maintenance in April 2014. The laboratory once more became operational in early June 2014.

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

Inger Damon, Victoria Olson, Irina Gates, Ashley Kondas, Laura Hughes-Baker, Subbian S. Panayanampalli

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

This protocol was last renewed in February 2014 and is valid through to December 2014.

The ability to validate nucleic acid-based and protein-based diagnostic capacity is critical for early detection and recognition of smallpox should a bioterror event result in reintroduction. The consequences of either false negatives, or false positives, will significantly impact our global public health system. “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 the variola DNA and variola antigen stocks at the WHO Collaborating Centre (WHO CC) for Smallpox and other Poxvirus Infections is important for future diagnostic development and validation.

DNA diagnostics

In 2010, several strains of cowpox virus showed cross reactivity to a previously validated variola-specific signature (VRL1). Identification and characterization of unique variola virus signature sequences have been assessed for specificity and sensitivity to variola virus. Assessment of sensitivity has used non-infectious variola virus nucleic acid from the DNA repository. Together with the United States Laboratory Response Network (LRN) Technical Review Committee and FDA, these assays are entering final stages of validation, having completed a multi-centre reproducibility study. The multi-centre study utilized a plasmid containing noncontiguous assay target fragments (<500 bp each) of variola virus nucleic acid. The LRN will be working with CDC in evaluating these data in preparation of submitting the diagnostic assays for FDA approval.

Protein diagnostics

Since late 2011, studies have been continuing on monoclonal antibody characterization, viral antigen capture assays, protein microarray development and novel methods for high throughput viral neutralization assays applicable to variola virus. This past year, work has focused on standardizing virus preparations, re-subcloning promising hybridomas, and working on bioinformatics-guided approaches to variola-specific monoclonal antibody design. Several peptide-derived monoclonals have been screened for reactivity against gamma-irradiated variola virus samples and specificity determined in comparison with other orthopoxviruses. We are currently optimizing a variola virus encoded protein microarray.

ASSESSMENT OF THE NEUTRALIZING ACTIVITY OF VACCINATED HUMAN VOLUNTEERS BLOOD SERA AND THOSE OF IMMUNIZED LABORATORY ANIMALS USING LIVE VARIOLA VIRUS

Ar.A. Sergeev, Al.A. Sergeev, A.S. Kabanov, A.S. Zamedyanksya, K.A. Titova, R.A. Maksyutov, S.N. Shchelkunov

State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, Russian Federation

In the course of research conducted in 2013-2014, an assessment of smallpox antibody levels in the sera of human volunteers vaccinated with the Revax-BT vaccine and in the sera of guineapigs immunized with a cell-based recombinant microencapsulated smallpox vaccine (CRMS vaccine) was performed as well as an assessment of the smallpox antibody levels in the sera of mice immunized with a genetically modified cell-based smallpox vaccine (GMCS vaccine) produced on the basis of the L1VP vaccinia virus strain with five deleted pathogenicity genes. The investigations demonstrated the smallpox antibodies to appear in the sera in 100% of cases.

Inoculation with the CRMS vaccine as well as the GMCS vaccine induces the formation of the specific antibodies in the immunized animals that neutralize variola virus (VARV). The level of the antibodies in the blood of the immunized animals is comparable to the level of the antibodies in the animals immunized with the L1VP vaccinia virus strain that is part of the conventional live smallpox vaccine for dermal administration.

DISCOVERY OF NEW ANTIVIRALS FOR SMALLPOX TREATMENT AND PREVENTION

Ar.A. Sergeev, A.S. Kabanov, Al.A. Sergeev, L.N. Shishkina, N.N. Bormotov, K.A. Titova, A.S. Zamedyanskaya, L.E. Bulychev, A.P. Agafonov, A.N. Sergeev

State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, Russian Federation

In 2013, SRC VB VECTOR tested about 70 new chemically synthesized compounds of the “NIOCH”, “Acad” and “Perm” series against surrogate orthopoxviruses (vaccinia virus, cowpox virus, and ectromelia virus). For the purpose of further research, 19 compounds were selected that showed greater antiviral effects, i.e. 15 compounds of the NIOCH series, 3 compounds of the Acad series, and 1 compound of the Perm series. In 2014, the antiviral activity of these chemical compounds was tested against the Ind-3a strain of variola virus in vitro.

Four compounds, representing complex compounds of functional derivatives of pyrrolidine-2,5-dione with nickel, show high anti-orthopoxviral activity but are still inferior in activity to NIOCH-14 and ST-246. Among compounds representing other classes of chemical compounds examined to date, no other highly potent compounds against VARV were discovered.

In this regard, it is imperative to continue research in order to discover any chemical compounds of different classes with the potential to be developed as drugs against orthopoxviruses.

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

Inger Damon, Nadia Gallardo-Romero, Christina Hutson, M. Shannon Keckler, Johanna Salzer, Scott Smith, Paul Hudson, Darin Carroll, Victoria Olson

WHO Collaborating Center for Smallpox and other Poxvirus Infections, Atlanta, GA, United States of America

A number of animal models of systemic orthopoxvirus disease have been developed to evaluate various safer smallpox vaccines or therapeutics. The majority of these models have short disease incubation periods, which do not resemble that which is seen in human smallpox. As a result, these animal models are difficult to use to evaluate the use 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.

Historically, laboratory research efforts have tested several animal species for susceptibility to variola virus, but as yet, non-human primates are the only other 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 - 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.

In general, analogous to variola virus in non-human primates, inbred mouse strains are relatively difficult to infect, and obtain symptomatic illness, with monkeypox virus. A study surveying a large panel of inbred mouse strains has identified a strain (CAST/EiJ) which is highly susceptible to infection with monkeypox virus (Americo, J. L., B. Moss, P.L. Earl. 2010. Identification of wild-derived inbred mouse strains highly susceptible to monkeypox virus infection for use as small animal models. *J. Virol.* **84**(16):8172-8180). Unpublished data from the same laboratory has suggested that CAST/EiJ mice are highly susceptible to a range of orthopoxviruses (the genus of poxvirus to which both variola and monkeypox viruses belong) at lower infectious doses than seen in other inbred mouse strains. The potential utility of a rodent challenge model using variola virus – supplied from inbred populations with minimal intrinsic variability, greater availability of specific immunologic reagents, and ease of animal handling – makes it of great interest to determine if they are susceptible to disease.

We intranasally challenged CAST/EiJ female mice (5-6 weeks old), obtained from Jackson Laboratories (Bar Harbor, ME) with a range of doses of variola virus (5×10^3 – 5×10^6 plaque forming units). Mice were co-housed in groups of 3-5 animals per ventilated cage in the High Containment Laboratory (BSL 4). Standard mouse husbandry practices were performed during the experiment in accordance with CDC Institutional Animal Care and Use Committee (IACUC) guidelines. In addition to mouse chow all animals received treats as appetite monitors, as well as a plastic nests and other enrichment materials. Daily observations of the animal's food consumption, activity level and general appearance were recorded. Clinical criteria were used to assess for euthanasia criteria. Three times a week body weights were recorded, complete skin examinations were undertaken, and oral swabs were collected while

under anaesthesia with 3-5% isoflurane gas. One of the five animals in each of the 10^5 and 10^3 pfu challenged groups died/were euthanized during the study (day 15 and 16 post infection, respectively). At day 21 post infection the remaining animals were humanely euthanized for necropsy. All animals challenged with live virus seroconverted and several animals shed virus in the oropharynx between days 7-11 post infection. Viable virus from oropharynx or lesion was <220 pfu/swab. The clinical signs observed were decreased activity, ruffled hair, conjunctivitis and very few tail lesions; animals did not exhibit weight loss greater than that observed in the gamma-irradiated variola virus challenge group.

USE OF LIVE VARIOLA VIRUS TO EVALUATE ANTIVIRAL AGENTS SMALLPOX

Victoria Olson, Paul Hudson, Ashley Kondas, Zachary Reed, Scott Smith, Inger Damon

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External collaborators: John Connor, Boston University School of Medicine; Randall Lanier, Chimerix

This protocol was last renewed in February 2014 and is valid through to December 2014.

The presentation will also include updates from the proposal “Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention” in collaboration with Jason Kindrachuk and Peter B. Jahrling NIH/NIAID Integrated Research Facility, Frederick, MD, United States of America

The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox somehow re-emerges. Thus, 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. Considerable progress has been made on advanced development of two compounds. One compound, ST-246 has been procured by the US government and is available for use through an investigational new drug protocol via the US strategic national stockpile. The second compound is CMX-001, the orally available derivative of cidofovir, which functions as a nucleotide analogue to inhibit viral DNA replication. Work with multiple strains of variola virus containing various non-synonymous coding changes in the viral drug target, the DNA polymerase, established the EC₅₀ of CMX-001 remained ~100 times more potent than cidofovir. This work was recently published in *Antimicrobial Agents and Chemotherapy* [2014 Sep; **58**(9):5570-5571].

The Advisory Committee has continued to support the evaluation of new compounds, given the uncertainties of the drug development process. This project focused specifically on evaluation of antiviral efficacy, or mechanism of action, against live variola virus. Compounds specifically targeting viral proteins, viral processes or cellular functions required by the virus but non-essential for the human host are presently of great interest. Critical steps to evaluate such therapeutics require in vitro and/or in vivo animal model characterization of their activity against live variola virus infection.

The manuscript by Dower, *et al.* [*J Virol.* 2012 Mar; **86**(5):2632-40] recently identified CMLDBU6128 as an inhibitor of vaccinia, cowpox, and monkeypox viruses replication. The compound (IC₅₀ ~5 µmol/l against vaccinia virus) appears to inhibit the poxviral DNA-dependent RNA polymerase. This polymerase is a core component of many poxviruses and is highly conserved between vaccinia, cowpox, monkeypox, and variola viruses. Though CMLDBU6128 targets the viral polymerase, it is not functioning as a nucleotide analogue, as in vitro assays show that CMLDBU6128 directly inhibits RNA synthesis of intermediate and

late genes. This demonstrates that the mechanism of action of this drug is different than that of other antipoxviral compounds. A transcription inhibitor may be of significant therapeutic benefit to inhibit expression of poxvirus immunomodulatory proteins. This presentation will briefly update work on the evaluation of pyridopyrimidinone inhibitory compounds for activity against variola virus.

Finally, there is an increasing appreciation that many host responses are regulated by kinase-mediated phosphorylation events independent of changes in transcription or translation. Thus, global investigations of the activation state of host kinases, i.e. the kinome, provides a functional mechanism for identifying host signalling networks or individual kinases that are altered during the course of infection. Several studies have demonstrated that pharmacological targeting of cellular processes may inhibit variola virus multiplication and enable prophylaxis. Our research proposal utilizing global kinome analysis identified potential host therapeutic targets, and provided novel information regarding the molecular mechanisms of variola virus pathogenesis. Identification of novel host therapeutic targets may allow “repurposing” of drugs, currently cleared by the Food and Drug Administration (FDA) for other indications, in the treatment of orthopoxvirus infection. This presentation will present our preliminary evaluation of effectiveness of certain approved compounds (FDA approved for other indications) to halt variola virus multiplication.

DEVELOPMENT OF ANIMAL MODELS TO STUDY THE EFFICACY OF THERAPEUTIC AND PREVENTIVE PRODUCTS AGAINST SMALLPOX

L.E. Bulychev, A.I.A. Sergeev, A.S. Kabanov, Ar.A. Sergeev, O.V. Pyankov, S.A. Bodnev, D.O. Gorbatovskaya, A.S. Zamedyanskaya, N.I. Bormotov, L.N. Shishkina, S.N. Shchelkunov, A.P. Agafonov, A.N. Sergeev

State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, Russian Federation

This report provides data from a study to assess the possibility of using ICR mice and immunodeficient inbred SCID mice (Charles River), when infected intranasally with variola virus (VARV), as a laboratory model of smallpox designed to test the therapeutic and prophylactic and/or antiviral action of investigational new drugs. The sensitivity of mice to VARV has been established, i.e. a 50% infecting dose ($_{LID_{50}}$), as measured by the presence of the virus in the lungs of mice following 3 days (for ICR mice) and 4 days (for SCID mice) post infection, which made 2.7 ± 0.4 log PFU and 3.5 ± 0.7 log PFU, respectively. The rates of VARV accumulation in the organs and tissues of the intranasally infected ICR mice and SCID mice have been investigated: the virus has been detected in the brain, the nasal septum and in the lungs of ICR mice and in the nasal septum, trachea and in the lungs of SCID mice. The maximum virus concentrations in the lungs (3.8-4.1 log PFU/ml) in both ICR mice and SCID mice were observed following 2, 3, and 4 days post infection.

The virus accumulation within the lungs allowed using ICR mice to evaluate the activity of the NIOCH-14 lead compound. In mice treated with NIOCH-14, the virus titers in the lungs were significantly lower than in the mice in the control group. In addition, the virus was not detected, at all, in the lungs of four mice.

Thus, as a result of the experimental studies, it has been demonstrated that ICR mice can be used to assess the efficacy of anti-smallpox drugs.

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

Victoria Olson, Scott K. Smith, Paul Hudson, Ashley Kondas, Zachary Reed, Christine Hughes, Whitney Davidson, Inger Damon

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Additional external collaborators: Ichiro Kurane, National Institute of Infectious Diseases, Tokyo

This protocol was last renewed in February 2014 and is valid through to December 2014.

Variola virus neutralization in vitro remains an informative surrogate measure of smallpox vaccine efficacy. Our prior studies, using sera from vaccinia virus-vaccinees, have indicated that neutralization endpoint titers may differ between target viruses (variola virus - heterologous target versus vaccinia virus - homologous target). Slight differences in orthopoxvirus antigenic make-up likely account for these differences. 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, a vaccinee serum's ability 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**:2915-2931].

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 measure of efficacy. The role of variola virus neutralization as a marker for vaccine efficacy is valuable for the evaluation of vaccines that do not elicit a “take”, the traditional measure of vaccine success.

This presentation will update results from collaboration with 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), different strains of variola virus (representing both primary clades), and different vaccines (Dryvax versus LC16m8). We have also initiated discussions on how to determine neutralizing capacity of MVA vaccinee sera against variola virus. Initial steps will provide data on the dynamic range and reproducibility of our traditional PRNT assay.

These data will be essential for future submissions towards regulatory approval for these “third” generation vaccines.

SPECIES-SPECIFIC IDENTIFICATION OF VARIOLA, MONKEYPOX, COWPOX AND VACCINIA VIRUSES BY MULTIPLEX REAL-TIME PCR ASSAY

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A method of one-stage rapid identification of variola (VARV), monkeypox (MPXV), cowpox (CPXV), and vaccinia (VACV) viruses, pathogenic for humans, utilizing a multiplex real-time TaqMan PCR (MuRT-PCR) assay was developed. Four pairs of oligonucleotide primers and four hybridization probes with various fluorescent dyes and the corresponding fluorescence quenchers were concurrently used for the MuRT-PCR assay. The hybridization probe specific for the VARV sequence contained FAM/BHQ1 as a dye/quencher pair; MPXV-specific, TAMRA/BHQ2; CPXV-specific, JOE/BHQ1; and VACV-specific, Cy5/BHQ3. The specificity and sensitivity of the developed method was assessed by analysing DNAs of many strains belonging to six orthopoxvirus species as well as the DNA samples isolated from archive clinical specimens of human smallpox cases and experimental specimens isolated from CPXV-infected mice and MPXV-infected marmots.

PCR BASED DIAGNOSTIC ASSAY FOR THE MULTIPLEX DETECTION OF VARIOLA AND AGENTS OF VIRAL HEMORRHAGIC FEVER: THE NEXT PHASE

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The exponential epidemic of a CDC designated category A pathogen anywhere in the world poses a serious threat to our international security. These agents include viruses that cause viral haemorrhagic fever (VHF) syndrome as well as variola, the agent of smallpox. VHF is characterized by haemorrhage and fever with multi-organ failure leading to high mortality and morbidity. Smallpox, a prior scourge, has been eradicated for decades making it a particularly serious threat if released nefariously in the essentially non-immune world population. As the current epidemic of Ebola virus in West Africa has highlighted, early detection of the causative agents and ability to distinguish them from other pathogens is essential to contain outbreaks, implement proper control measures and prevent morbidity and mortality. We have been active in developing multiplexed detection assays that use species-specific PCR followed by ligase detection reactions. The resultant fluorescently-labelled ligation products have been detected on a universal array or bead express instrument, enabling the simultaneous detection and identification of multiple pathogens. Our assay was evaluated on 53 different isolates associated with VHF (Ebola virus, Marburg virus, Crimean-Congo hemorrhagic fever virus, Lassa fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus) as well as variola and vaccinia (the agent of smallpox and its vaccine strain, respectively). The assay was able to detect all viruses tested including eight sequences representative of different variola virus strains from the CDC repository. It does not cross-react with other emerging zoonotic viruses such as monkeypox virus or cowpox virus, or six flaviviruses tested (St. Louis encephalitis virus, Murray Valley encephalitis virus, Powassan virus, tick-borne encephalitis virus, West Nile virus and Japanese encephalitis virus). These multiplexed assays are poised for transitioning to high-throughput real-time format to provide accurate viral load and identification in the urban setting. In addition, we are developing point-of-care diagnostics (e.g. powered by cell phones) for use on the front lines.

UPDATE ON THE ATTENUATED SMALLPOX VACCINE LC16m8

Hiroyuki Yokote

General Manager Development Department, Vaccine Division, The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN), Kumamoto, Japan

LC16m8 is an attenuated replication-competent vaccinia virus and was developed from the Lister strain by serial passaging in primary rabbit kidney cells in the 1970s. LC16m8 has low neurovirulence and shows a good protective efficacy in animal models. Based on the clinical data obtained during its development stage, the LC16m8 vaccine was licensed in Japan in 1975. The LC16m8 vaccine has been given to approximately 90 000 infants and more than 8000 members of the armed forces in Japan without any severe adverse effects. Currently, the LC16m8 vaccine is intended for an emergent use and has been stockpiled against potential bioterrorism with smallpox virus in Japan since 2001.

The LC16m8 vaccine is a lyophilized product and has been confirmed to have a high stability. Current authorized shelf-life is four years at -20 °C storage however an ongoing stability study indicates a possible extended shelf-life. In addition, we obtained five-year stability data on drug substance of the LC16m8 vaccine. This study was driven by the need to respond to a demand of higher amounts of the vaccine. Typically, it takes six to nine months from an order up to a delivery of the vaccine, if the vaccine is manufactured from the beginning with raw materials. On the other hand, if the vaccine is manufactured from the drug substance, the duration required up to a delivery will be shortened to three to four months. Currently, the LC16m8 vaccine has become widely known and captured attention of relevant parties worldwide. The stock of the drug substance will be able to manage an emergent demand from overseas within a satisfactory duration.

CANADA APPROVES THE NON-REPLICATING SMALLPOX VACCINE IMVAMUNE® (IMVANEX®)

Dr Nathaly Arndtz-Wiedemann

Vice President Clinical R&D, Bavarian Nordic

In 2013 the MVA-based non-replicating smallpox vaccine achieved two registrations: in July by EMA under the trade named IMVANEX® (MVA-BN) the authorization covers active immunization against smallpox disease for the general adult population, including people with weakened immune systems (people diagnosed with HIV or atopic dermatitis) and in November by Health Canada under the trade name IMVAMUNE® for persons of 18 years of age and older who have contraindications to traditional replicating vaccines, including people with immune deficiencies and skin disorders. In November 2013 WHO include the non-replicating MVA vaccine in its stockpile.

IMVANEX® (IMVAMUNE® in the USA) is a live, highly attenuated vaccinia strain vaccine that does not replicate in human cells and has been developed as a stand-alone smallpox vaccine. IMVANEX® has been tested in more than 7500 subjects including 1000 subjects from risk groups with contraindications for replicating smallpox vaccines, i.e. HIV-infected and atopic dermatitis patients. IMVANEX® has in 20 completed or on-going clinical trials demonstrated a favourable safety profile in healthy individuals as well as in populations with impaired immune function and active atopic dermatitis.

IMVAMUNE® is currently part of the United States Strategic National Stockpile of medical products and a further four million doses were delivered to the stockpile in 2014 in addition to the 24 million already delivered. Other countries have also started to implement IMVANEX® in their preparedness after approval. In August 2014 both the Canadian health authorities and military upgraded their preparedness with IMVANEX®.

The broad approval of IMVANEX® provides a unique opportunity to update current preparedness plans, as current stockpiled replicating vaccines, does not allow for vaccination of all individuals in our societies. Between 5% and 25% of a given population are not considered candidates to receive replicating vaccines; this includes among others people with impaired immune systems and atopic dermatitis. The approval influences the ability to vaccinate first line responders and military in a pre- and post-event setting, as replicating vaccines are associated with a high rate of severe side effects, leaving compliance low and poses a severe cost benefit challenge.

TECOVIRIMAT:- UPDATE ON DEVELOPMENT AND DELIVERY

*Lek Chinsangaram, Annie Frimm, Shantha Kumar, Kady Honeychurch, Doug Grosenbach, Akile Berhanu, Biswajit Maiti, Laura Linscott and Dennis E. Hruby**

SIGA Technologies, Inc., 4575 SW Research Way, Corvallis, OR, United States of America

The antiviral drug tecovirimat has been under development for use to treat or prevent infections caused by orthopoxviruses, including variola virus, since 1983. The drug has been demonstrated to be safe, non-toxic, and efficacious in a large number of animal trials. In response to changing regulatory statutes, efforts are continuing to develop an appropriate data package to support regulatory approval under the “animal rule”. Updates on our regulatory progress as well as manufacturing and delivery of the drug to the United States’ Strategic National Stockpile will be provided.

UPDATE ON THE DEVELOPMENT OF BRINCIDOFIVIR (CMX001) FOR SMALLPOX AND OTHER INDICATIONS

Lawrence C. Trost

Chimerix Inc., Durham, NC, United States of America

Brincidofovir (CMX001) is a potent inhibitor of multiple dsDNA viruses including adenovirus, cytomegalovirus (CMV) and variola major. The key attributes of brincidofovir compared with intravenously administered cidofovir (vistide) are improved ease of use, oral bioavailability, increased potency and decreased nephrotoxicity. In addition, brincidofovir has demonstrated a favourable resistance profile and a low risk of significant drug-drug interactions. It has been administered to more than 1000 human subjects, many with life-threatening illnesses caused by dsDNA viruses. It has also been studied in patients with renal and/or hepatic impairment and pediatric subjects as young as 1 month of age. A Phase 3 study for prevention of CMV in Immunocompromised transplant patients is currently enrolling using doses that, scaling from efficacious doses in animal models, are also proposed for treatment of smallpox. The FDA has agreed that the intradermal rabbitpox model in rabbits and the intranasal ectromelia model in mice are acceptable animal models to support development of brincidofovir for a therapeutic indication for treatment of smallpox. FDA and Chimerix have further agreed on the design of a pivotal study in the RPXV model which will commence in late 2014. In addition, a large, blinded study of the efficacy of brincidofovir in the ECTV model is scheduled to begin in 2014. In August of 2014, BARDA exercised an option to provide approximately US\$ 17 million in additional funding to further advance the development of brincidofovir for treatment of smallpox. Supported by the work of BARDA's "Animal Model Development Program," Chimerix completed pharmacokinetic studies of brincidofovir in rabbits and mice that support the feasibility of scaling from efficacious doses in the animal models to the proposed human doses for treatment of smallpox. Brincidofovir is available in tablet and liquid formulations. Manufacturing of brincidofovir has been validated at commercial scale and the drug is stable for multiple years. Taken together the smallpox animal model and human efficacy data against related viruses support the likely efficacy of brincidofovir for treatment of smallpox.

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

FDA PROGRESS ON FACILITATING THE DEVELOPMENT AND APPROVAL OF SMALLPOX MEDICAL COUNTERMEASURES

Luciana Borio

Assistant Commissioner for Counterterrorism and Emerging Threats, Food and Drug Administration, Silver Spring, MD, United States of America

The Food and Drug Administration (FDA) in the USA is responsible for ensuring the safety, effectiveness, and security of medical products, including medical countermeasures (MCMs). FDA also works to help foster the development of MCMs—with the goal of achieving FDA approval¹—as well as facilitating timely access to MCMs in the event of a public health emergency.

The federal Government of the USA is supporting the development of smallpox MCMs, including drugs, vaccines, and diagnostic tests. This presentation highlights the regulatory progress made since the fifteenth meeting of ACVVR in 2013.

FDA's regulatory mechanisms (e.g., approval or emergency use authorization) for enabling access to MCMs during public health emergencies are based on benefit/risk assessments anchored in scientific evidence. Smallpox MCMs 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 are not available.

Regulatory uncertainties related to smallpox MCMs reflect scientific uncertainties. Diagnostics, drugs, and vaccines each present their own unique set of scientific uncertainties. FDA is working very closely with MCM developers – through mechanisms such as interactive review – to guide the development of smallpox MCMs and establish feasible and appropriate regulatory pathways for their approval. The focus of FDA's interactions with MCM developers is on providing feedback on proposed studies to support safety, pharmacokinetic, and animal model efficacy studies for antiviral drugs and pivotal efficacy studies for the attenuated vaccine. Measurable progress has been made and smallpox MCMs continue to advance in development.

¹ 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.

CHARACTERIZATION OF VIALS FOUND AT THE NATIONAL INSTITUTES OF HEALTH BETHESDA CAMPUS

Darin Carroll, Victoria Olson, Ashley Kondas, Yu Li, Zachary Reed, Scott Smith, Paul Hudson, Inger Damon

WHO Collaborating Center for Smallpox and other Poxvirus Infections, Atlanta, GA, United States of America

Prior to the smallpox eradication campaign, variola virus, the causative agent of smallpox, was routinely studied in multiple laboratories at lower biosafety levels. Forgotten glass ampoules storing some of these historic samples were found within refrigerated space at the National Institutes of Health Bethesda campus some 60 years after generation. The samples were carefully inventoried by staff from the Centers for Disease Control and Prevention (CDC) and all unknown samples or those designated as variola virus were transferred to the CDC biosafety level 4 laboratory for processing and characterization. Real-time PCR confirmed the presence of variola virus DNA within the six vials labeled variola virus or alastrim. These samples contained viable virus and underwent full genomic sequencing. The virus isolates generated four sequences that matched previously sequenced isolates as well as two unique sequences. The new sequences expand our knowledge on the diversity of variola virus strains. The samples are currently secured within the CDC biosafety level 4 laboratory awaiting destruction witnessed by a World Health Organization representative.

UPDATE ON VARIOLA VIRUS REPOSITORY BIOSAFETY INSPECTION

Kazunobu Kojima

World Health Organization (HSE/GCR), Geneva, Switzerland

The World Health Assembly has adopted a series of resolutions for the post smallpox eradication era, including resolution WHA60.1 in 2007 that specifies WHO's mandate to inspect the two WHO Collaborating Centre repositories, namely the Centers for Disease Control and Prevention (CDC) in the United States of America and the State Research Centre of Virology and Biotechnology (VECTOR) in the Russian Federation. This resolution requests the Director-General "to maintain [biennial] inspections of the two authorized repositories in order to ensure that conditions of storage of the virus and of research conducted in the laboratories meet the highest requirements for biosafety and biosecurity ... inspection mission-reports should be available for public information after appropriate scientific and security redaction".

The last inspections were carried out in 2012 and this year falls in the two-year cycle. Communications have been made with the two repositories, resulting in informal concurrence that the onsite inspections take place for VECTOR in December 2014 and CDC in May 2015. The timing of the visit was synchronised to the period when each facility in question is 'cold' or decontaminated for a scheduled maintenance.

A group of experts with different background is identified to complementarily cover all the expertise necessary to accomplish this mission in a team led by WHO. In the last inspections in 2012, the WHO's protocol based on the published international biorisk management normative document – a consensus Workshop Agreement registered with the European Committee for Standardization (CEN) CWA 15793 (2011) – was used as the basis of inspection. This methodology was thoroughly reviewed by the identified inspectors aiming at better contextual alignment with clarity.

A preparatory meeting is scheduled back-to-back with this ACVVR meeting with a participation of the repository representatives and the inspectors, in which the refined approach will be presented, discussed and expectedly agreed in advance to the onsite inspection. Planning and inspection procedures will also be discussed taking advantage of this opportunity.

Annex 2. Agenda

Sixteenth Meeting of the WHO Advisory Committee on Variola Virus Research

20 and 21 October 2014

**Salle A, WHO Headquarters
Geneva, Switzerland**

20 October 2014

9:00 - 9:15 S. Briand, Director, Pandemic and Epidemic Diseases

Election of chair

Variola virus reports

9:15 – 9:45 Report of the Secretariat - WHO Secretariat – A. Costa

9:45 – 10:00 Update on research proposals submitted to WHO in 2014 – R. Drillien

10:00 – 10:10 Report on the variola virus collection at the WHO Collaborating Centre Repository in VECTOR, Koltsovo, Novosibirsk, Russian Federation – A. Agafonov

10:10 – 10:15 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

Variola virus research 2012–2013 Update

10:15 – 10:45 Update on the development of nucleic acid based and protein based variola virus diagnostic assays (abstract title: Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support) – V. Olson

10:45 – 11:15 **Tea/coffee break**

11:15 – 11:35 Discovery of new antivirals for smallpox treatment and prevention/Assessment of the neutralizing activity of vaccinated human volunteers' blood sera and those of immunized laboratory animals using live variola virus – S. Shchelkunov

11:35 – 11:55 Use of live variola virus to determine whether CAST/Eij mice are a suitable animal model from human smallpox – I. Damon

WHO Advisory Committee on Variola Virus Research
Report of the Sixteenth Meeting, Geneva, Switzerland, 20 and 21 October 2014

- 11:55 – 12:15 Use of live variola virus to evaluate antivirals against smallpox/
Characterizing Variola Pathogens through System Kinomics – V. Olson
- 12:15 – 12:35 Development of animal models to study the efficacy of therapeutic and
preventive products against smallpox – A. Agafonov
- 12:35 – 14:00 **Lunch**
- 14:00 – 14:20 Use of live variola virus to support less-reactogenic vaccine development:
continued evaluation of third-generation vaccines – V. Olson
- 14:20 – 14:40 Species-specific identification of variola, monkeypox, cowpox, and
vaccinia viruses by multiplex real-time PCR assay – S. Shchelkunov
- 14:40 – 15:00 Novel diagnostic assay development process - L. Golightly,
15:00 – 15:20 Update on LC16m8 vaccine – H. Yokote
- 15:20 – 15:50 **Tea/coffee break**
- 15:50 – 16:10 Update on smallpox vaccine IMVANEX® (IMVAMUNE®) – N. Arndtz-
Wiedemann
- 16:10 – 16:30 Progress towards approval and deployment of Arestvyr® (ST-246) –
D. Hruby
- 16:30 – 16:50 Update on the development of brincidofovir (CMX001) for smallpox – L.
Trost
- 16:50 – 17:10 FDA progress on facilitating the development and approval of smallpox
medical countermeasures – L. Borio
- 17:10 – 17:30 Recent found vials at NIH, USA – D. Carroll
- 17:30 – 17:40 Update: variola virus repositories biosafety inspection visits - WHO
- 17:40 – 18:30 General discussion

DAY ONE CLOSE

21 October 2014

- 9:00 – 10:30 Discussion on future variola virus research
- 10:30 – 11:00 **Tea/coffee break**
- 11:30 – 12:30 General discussion and preparation of draft meeting report

WHO Advisory Committee on Variola Virus Research
Report of the Sixteenth Meeting, Geneva, Switzerland, 20 and 21 October 2014

12:30 – 13:30 **Lunch**

13:30 – 16:00 Final discussion and finalization of draft report

SIXTEENTH ACVVR MEETING CLOSES

Annex 3. List of participants

Sixteenth Meeting of the WHO Advisory Committee on Variola Virus Research

20 and 21 October 2014

Salle A, WHO Headquarters, Geneva, Switzerland

MEMBERS OF THE ADVISORY COMMITTEE

Dr Alexander Agafonov, Deputy Director-General, Federal State Research Institution, State Research Center of Virology and Biotechnology (VECTOR), Federal Service for Surveillance on Consumer Rights Protection and Human Well-being, Koltsovo, Novosibirsk Region, RUSSIAN FEDERATION

Dr Beth Bell, Director National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, UNITED STATES OF AMERICA

Dr Robert Drillien, Directeur de Recherche, Institut de génétique et de biologie moléculaire et cellulaire, Illkirch Cedex, FRANCE

Professor Mariano Esteban, Director, Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología, Madrid, SPAIN

Dr David Evans, Professor and Vice-Dean, Research, Faculty of Medicine & Dentistry, Mackenzie Health Sciences Centre, University of Alberta, Edmonton, CANADA

Dr Richard J. Hatchett, Chief Medical Officer and Deputy director, Biomedical Advanced Research and Development Authority (HHS/ASPR/BARDA), Washington, DC, UNITED STATES OF AMERICA

Dr George W. Korch, Senior Science Advisor, Office of the Assistant Secretary for Preparedness and Response, Department of Health and Human Services, Washington, DC, UNITED STATES OF AMERICA

Professor J. Michael Lane, Professor Emeritus of Preventive Medicine, Emory University School of Medicine, Atlanta, Georgia, UNITED STATES OF AMERICA

Dr Andreas Nitsche, Head of Division, Highly Pathogenic Viruses, Centre for Biological Security, Robert Koch-Institut, Berlin, GERMANY

Professor Pilaipan Puthavathana, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, THAILAND

Dr Anthony J. Robinson, Virologist, Michelago, NSW, AUSTRALIA

Dr Li Ruan, Professor, Biotech Center for Viral Disease Emergency, Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, CHINA

Professor Geoffrey L. Smith, President, International Union of Microbiological Societies; Wellcome Trust Principal Research Fellow; Head, Department of Pathology, University of Cambridge, Cambridge, UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

Dr Oyewale Tomori, Regional Virologist for the WHO African Region, Oyo State, NIGERIA

Dr Henda Triki, Chief, Laboratory of Clinical Virology, Institut Pasteur de Tunis, Tunis, TUNISIA

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Dr Antonio Alcami, Research Professor, Centro de Biotecnología Molecular Severo Ochoa (CSIC-UAM), Campus de Cantoblanco, Madrid, SPAIN

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Dr Luciana L. Borio, Assistant Commissioner for Counterterrorism Policy, Food and Drug Administration, Silver Spring, Maryland, UNITED STATES OF AMERICA

Dr Darin Carroll, Acting Chief, Poxvirus Section and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, UNITED STATES OF AMERICA

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Dr Manuela Mura, Scientific Officer, Anti-Infectives and Vaccines, Scientific and Regulatory Management, European Medicines Agency, London, UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

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Dr Masayuki Saijo, Director, Department of Virology 1, National Institute of Infectious Diseases, Tokyo, JAPAN

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Dr Dennis E. Hruby, Chief Scientific Officer, SIGA Technologies Inc., Corvallis, Oregon,
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Mr Alejandro Costa, Team Lead, CED/EVS

Dr Pierre Formenty, Team Lead, CED/EZD

Dr Keiji Fukuda, ADG/HSE

Dr William Perea, Coordinator, PED/CED

Dr Isis Pluut, Technical Officer, PED/CED

Dr Kazunobu Kojima, Scientist, GCR/CAD