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WHO Advisory Committee on Variola Virus Research

Report of the Fourteenth Meeting

Geneva, Switzerland
16–17 October 2012

World Health Organization

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Executive summary

Variola virus research performed under supervision of WHO was presented to the WHO Advisory Committee on Variola Virus Research. It may be summarized as follows.

The Committee noted that the work under the authorized programme of research with variola virus had been done under its supervision. In 2012, nine projects had been approved by its scientific subcommittee and progress reports were presented at the meeting.

The Committee received reports on the virus collection held at the two WHO Collaborating Centres authorized as repositories of variola virus: the State Research Center for Virology and Biotechnology (Russian Federation) and the Centers for Disease Control and Prevention (United States of America).

In addition, the Committee received updates from three pharmaceutical companies on advanced candidate vaccines and antivirals. Information presented included data on efficacy, safety, stability and large-scale manufacturing capacity. Work is continuing on the studies that are needed in order to satisfy the requirements for eventual regulatory approval.

The Committee heard that limited progress had been made in establishing a laboratory network for diagnosis of smallpox and other orthopoxvirus infections due to a variety of logistic issues. WHO informed the Committee that Headquarters and Regional Offices will identify and select facilities with appropriate capacities for this purpose from existing diagnostic laboratories for dangerous pathogens.

A variola-virus-specific diagnostic test based on existing tests and with the capacity to distinguish between variola and other poxviruses is being refined.

Both authorized repositories of variola virus were inspected during 2012 and the final reports of these biosafety inspections will be posted on the WHO web site. The protocol used followed the European Committee for Standardization's Laboratory Biorisk Management Standard CWA 15793:2011 and addressed 16 elements of laboratory biorisk management.

1. Opening

1.1. The 14th meeting of the WHO Advisory Committee on Variola Virus Research (ACVVR) took place in WHO Headquarters, Geneva, Switzerland, from 16 to 17 October 2012 with Professor G.L. Smith as Chairman and Dr R. Drillien as Rapporteur. Dr K. Fukuda, WHO Assistant Director-General for Health Security and Environment welcomed participants on behalf of WHO. Dr Fukuda reminded the Committee of the 2007 World Health Assembly (WHA) resolution 60.1, which noted that authorization was granted to permit essential research for global public health benefit including further research into antiviral agents and safer vaccines. He thanked the ACVVR for conducting yearly reviews of the research programmes involving live variola virus. He reminded the Committee that nine proposals for research on live virus had been submitted and reviewed by the ACVVR scientific subcommittee since the last meeting and that progress in each area would be presented at this meeting. He informed the Committee that inspections of the two WHO Collaborating Centres had been carried out in 2012. The reports of these inspections would shortly be made publicly available. He also reminded the Committee that an operational framework for distributing the current vaccine stockpiles administered by WHO including both physical stocks in Geneva and vaccines pledged to WHO was being set up according to standardized operating procedures.

2. Report of the Secretariat

2.1. Dr A. Costa presented the report of the WHO Secretariat. He recalled that biosafety inspections of the two WHO Collaborating Centres had been carried out in 2012 at the Centers for Disease Control and Prevention (CDC), United States of America, during 7–11 May and at VECTOR, Russian Federation, during 3–10 October. He summarized the strategic preparedness plan that was being revised by WHO since the beginning of 2012 to deal with any smallpox outbreak. The plan included the handling of vaccine stockpiles, the creation of a diagnostic laboratory network, the identification of appropriate assays and the sharing of information between experts. He indicated that the Smallpox Laboratory Network would be included within the WHO dangerous pathogens network to ensure efficient use of resources. Dr Costa informed the Committee that WHO has consulted with the countries which pledged vaccines in order to ensure emergency coordination of, and access to, the WHO vaccine stockpile; and that this is an on-going process. The physical inventory of the stockpile is expected to be updated. Finally, the importance of the present meeting was underlined given the prospect of a discussion by the World Health Assembly in 2014 to set a date for destruction of variola virus stocks.

2.2. **Discussion:** The report by Dr Costa was followed by a question concerning the establishment of a checklist for the inspection of the Collaborating Centres. Dr Costa responded that the inspections had indeed been carried out according to a formally standardized procedure with checklists. Another comment from a Committee member stressed the need for knowledge of the actual physical stockpile under WHO responsibility, information that would be provided later in the meeting.

3. Update on research proposals submitted to WHO in 2011

3.1. Dr R. Drillien reported on the work of the scientific subcommittee in charge of reviewing research proposals on live variola virus submitted to WHO. Since the last ACCVR meeting in 2011, nine projects had been examined, the majority of which were requests for renewal of ongoing work at the two WHO Collaborating Centres. The subcommittee recommended that all nine projects be approved by WHO, with the exception of a portion of one project devoted to sequencing additional variola virus genomes. Further clarification was sought by the subcommittee regarding this project particularly with respect to whether amplification of variola virus stocks was required and whether the study involved distributing large segments of virus DNA to external laboratories.

3.2. **Discussion:** Questions were raised regarding the process followed by the scientific subcommittee to make their recommendations to WHO. Dr Drillien responded that the subcommittee had strived in their analyses to follow the overall recommendations and opinions of the Committee as a whole and that the main reason for the subcommittee was to avoid repeated consultations with the entire Committee between yearly meetings. The need for timely communication of WHO to the subcommittee on the final decisions on the research proposals it reviews was underlined.

4. Report on the variola collection at the WHO Collaborating Centre repository in SRC VB VECTOR, Koltsovo, Novosibirsk Region, Russian Federation

4.1. Dr A.N. Sergeev provided an update on the variola virus repository held at VECTOR. He reminded the Committee that VECTOR maintains a collection of 120 isolates of variola virus from Europe, Asia, Africa, South America and Eastern Mediterranean and that these samples were transferred in 2010 to a building designated for research on variola virus. The collection includes freeze-dried and frozen cultures as well as 17 primary specimens isolated in the past from human patients. The total number of registered samples is 696 units stored in cryovials. In 2012, the Ind-3a variola virus strain was grown in Vero cells and used to assess antiviral properties of chemically synthesized compounds and therapeutic antibodies. Research planned for 2013 will focus on discovering new antivirals, chemically synthesized compounds for treatment and prevention of smallpox, the development of smallpox therapeutic antibodies, the assessment of variola virus neutralizing activity of sera from those vaccinated against smallpox and the development of animal models to study the efficacy of therapeutic and preventive preparations against smallpox. Finally, it was recalled that the Collaborating Centre at VECTOR had been inspected by WHO in October 2012.

4.2. **Discussion:** In response to questions raised by the Committee, Dr Sergeev pointed out that only a small subset of the variola virus isolates was being used for research purposes at VECTOR (these isolates had been highlighted in his presentation) and that no removals of variola virus samples had occurred since the last meeting in 2011.

5. Report on the variola virus collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centres for Disease Control and Prevention Atlanta, GA, USA

5.1. Dr I. Damon provided an update on the variola virus CDC repository. She reminded the Committee that a new facility for research on variola virus had been functional since 2009 with regular interruptions for routine, preventive maintenance. The majority of the variola virus isolates at CDC were originally propagated on embryonated eggs and identified during the final years of the intensified smallpox eradication campaign. The virus collection is maintained in two separate freezers, one of which is a back-up freezer. Secure databases have been constructed to track the use of variola virus. Annual reports on the status of these collections have been provided to WHO. No new virus seed pools have been added to the inventory between 2011 and 2012. A working stock of purified variola virus Harper strain was prepared and stored as single use vials for animal studies carried out in 2012. Between November 2011 and September 2012 there were removals of variola virus or samples from prior studies from the repository for WHO-sanctioned protocols that are presented in this meeting's report.

5.2. One research focus was the acquisition of additional sequence data for variola virus isolates. During December 2011, original scabs or homogenates were processed for sequencing using Illumina technology after initial findings with monkeypox material showed that it was feasible to use very small amounts of infected tissue for DNA sequencing without the requirement for virus amplification. Application of this technology to four samples demonstrated that one was actually a vaccinia virus sample (due to generalized vaccinia mistaken for variola). Another variola virus sample clinically characterized as ordinary confluent smallpox surprisingly displayed a DNA sequence closest to the alastrim clade, which induces an epidemiologically distinct form of smallpox. Overall, the DNA sequences of large genomic regions of three variola virus samples were obtained and although no full length sequence could yet be assembled it was thought that this may be possible in the future. An outstanding feature of one sample, passaged 124 times in embryonated eggs, was the heterogeneity of the variola DNA sequences in distinct regions of the genome.

5.3. **Discussion:** The subsequent analysis of heterogeneity in the other deep-sequenced variola isolates will facilitate understanding of whether heterogeneity arose on amplification of virus on the chicken chorioallantoic membrane (CAM) (and/or cell culture) or whether virus quasi-species were already present in smallpox patients, another possible explanation. It was also underlined that the uncertainty of change introduced by CAM passaging may complicate the ability to trace the origin of a variola virus incident, should it occur, in a forensic study. A set of serially passaged isolates, currently in the WHO Collaborating Center freezers, may help understand the changes introduced by serial tissue culture passage in BSC-40 cells. Finally, it was noted by members of the Committee that the sequencing experiments had resulted in a small reduction in the size and/or number of total samples of variola virus held at the CDC.

Variola virus research 2011–2012 update

6. Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus / Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support

6.1. Dr I. Damon summarized the available methods for detection of variola virus using nucleic acid and protein based detection techniques. She pointed out that the nucleic acid diagnostic platforms for orthopoxviruses developed in previous years are no longer being supported by some companies, as newer platforms are been designed. This is one compelling reason to maintain the WHO Collaborating Center variola DNA stocks, and variola antigen stocks for continued diagnostic development. A confounding factor derives from the finding reported several years ago of a few cowpox virus isolates that display cross reactivity to a previously validated variola-specific signature. Recent assessment of specificity and sensitivity of variola virus nucleic acid detection methods at the CDC has allowed the identification of two new signatures that are in preclinical stages of development. These assays have used non-infectious, genomic variola virus nucleic acid from the DNA repository.

6.2. Studies designed to improve a variola virus capture method using monoclonal antibodies in a protein based detection assay were also presented. Preliminary experiments demonstrated that time resolved fluorescence assays could allow a considerable increase in the sensitivity of detection of orthopoxviruses (800 fold). The possibility of applying these techniques to variola virus would however depend on the availability of a species-specific monoclonal antibody for variola virus. One such monoclonal antibody has been identified in the past at the CDC Collaborating Center but it was found to recognize gamma-irradiated virus more efficiently than native virus and further studies were planned to examine whether this may be an artefact of the method.

6.3. Additional studies have continued on developing a high-throughput assay for evaluation of neutralizing antibodies against variola virus in a 96-well format where a primary anti-orthopoxvirus antibody is employed followed by a secondary antibody coupled to a fluorescent probe. So far the assay applied to vaccinia virus has displayed good reproducibility and correlated well with the traditional plaque reduction neutralization test (PRNT). It was proposed to apply this technique to variola virus in future studies.

6.4. **Discussion:** One Committee member suggested that a DAPI(4',6-diamidino-2-phenylindole)-based fluorescent assay could usefully substitute for the more cumbersome PRNT assay as a high-throughput method. Another member pointed out that if the anti-variola monoclonal antibody proved to be a sensitive tool for protein based diagnostics it was still uncertain whether all variola virus isolates would be recognized and therefore the tool may be insufficient. In response to questions concerning the identity of the variola antigen recognized by the variola-specific monoclonal antibody from the CDC, Dr Damon stated that the epitope recognized was conformational and had not yet been identified but that it was

possible, in view of preliminary data, that it may be comprised of a complex between more than one virus protein.

7. Discovery of new antivirals for smallpox treatment and prevention / Development of therapeutic anti-smallpox antibodies / Assessment of the neutralizing activity of vaccine blood sera using live variola virus

7.1. Dr L.E. Bulychev reviewed studies conducted at the Collaborating Centre at VECTOR to evaluate new antiviral treatments for variola. At the ACVVR meeting held in 2011, the anti-variola activity of the compound NIOCH-14 in tissue culture infected cells was reported and shown to be similar to a compound that precisely mimics ST-246®. It was also shown that infection of marmots (steppe marmot, species *Marmota bobac*) with monkeypox virus might provide a useful animal model for monkeypox virus since these animals displayed pronounced clinical signs of the disease.

7.2. In 2012, the therapeutic and prophylactic activity of NIOCH-14 as well as the ST-246® mimic were examined in marmots infected intranasally with the monkeypox virus strain, V79-1-005, at a dose of 30 ID_{50} ($4.5x10^3$ plaque-forming units [PFU]). Both drugs, administered orally one day before inoculation and then within 15 days following the inoculation at a daily dose of 40 mg/kg, completely suppressed clinical signs of disease. All animals that received treatment developed antibodies that neutralized live monkeypox virus. Two out of four animals in the untreated, infected group succumbed whereas all four infected, NIOCH-14 treated, animals survived monkeypox virus infection.

7.3. In addition, antiviral activity of 88 chemically synthesized drugs was evaluated against several orthopoxviruses and four of the most potent compounds in the initial assays were tested in vitro for their activity against the Ind-3a variola virus strain. One compound NIOCH-92 displayed the highest activity and could be promising although it was less effective in vitro than the ST-246® mimic.

7.4. Other studies have addressed the antiviral activity of humanized monoclonal antibodies against vaccinia virus that were produced in Chinese hamster ovary cells. These antibodies proved to be active against vaccinia virus in an enzyme-linked immunosorbent assay (ELISA), but were ineffective at neutralization.

7.5. Finally, the research programme designed to evaluate variola neutralizing antibody levels in the sera of individuals vaccinated with new variants of smallpox vaccines was not presented because this project was scheduled to start in the second half of 2012 and continue into 2013.

7.6. **Discussion:** In reply to one question about the chemical nature of the antiviral compound developed at VECTOR (NIOCH-14) and its mechanism of action, it was indicated that this compound is a structural analogue of ST-246® and that it is expected to have the same mechanism of action. It was also reported that a description of the anti-variola efficacy of this drug had been published in a scientific journal in Russian.

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

8.1. Dr I. Damon recalled recently published studies demonstrating that the inbred mouse strain CAST/EiJ is highly sensitive to monkeypox virus. This provided an incentive to check for sensitivity to variola virus infection with the possibility of establishing a small rodent model for smallpox. The variola virus Harper strain concentrated by pelleting through a 36% sucrose cushion was used to infect female CAST/EiJ mice aged 8–9 weeks. The virus was administered by the intranasal route over a range of doses ($5x10^2$ to $5x10^6$ PFU/animal) and the animals followed over a three-week period. Clinical signs (nasal and oral oedema, weight loss) and symptoms of illness (reduced grooming, reduced activity) were observed in animals infected with the higher viral doses but no animals succumbed. Viral shedding was documented in oral swabs and ocular secretions by polymerase chain reaction (PCR). Very few skin lesions were seen even at high viral doses. Evaluation of all available clinical parameters indicated that 8-week-old mice were more sensitive to the infection than 9-week-old animals. Follow-up work will be conducted on the sera of these animals to assay for antibody production and viraemia. The results demonstrate that the CAST/EiJ mice do not display the acute sensitivity to variola virus that was observed in previous work with monkeypox virus. It is postulated that the weak sensitivity of CAST/EiJ mice to variola virus may be due to the experimental design, which used animals older than those in the previously published monkeypox studies.

8.2. **Discussion:** It was suggested that it would be interesting to conduct sequential sampling of tissues to look for pathology or attempt different routes of infection if future experiments were to be performed. Another comment noted that CAST/EiJ mice may not be completely inbred and that enhanced sensitivity of some of the animals could correlate with specific histocompatibility antigens in a fraction of the mouse colony. The relevance of this animal model for smallpox was also questioned in view of the fact that the CAST/EiJ mice were recently reported to be sensitive to monkeypox virus because of a relative defect in the interferon gamma response, while an absolute defect of gamma interferon production would not be expected for most human beings.

9. Use of live variola virus to evaluate antiviral agents against variola

9.1. Dr V. Olson reminded the Committee of the goal set in previous years of the development of at least two anti-variola virus compounds with distinct mechanisms of action that should ultimately achieve licensure for use. She summarized the various targets in the viral life-cycle that had been identified and the drugs currently being evaluated. While the progress made with the two leading compounds is encouraging, consideration of the uncertainties of the drug development process has prompted exploratory work on alternative drug candidates in case either of the leading candidates fails. Data were presented on a proteasome inhibitor (MG-132) that has previously been shown to display antiviral activity by inhibiting the

formation of vaccinia virus replication factories, a step in the cycle unaffected by the leading compounds identified so far. Obtaining one of the proteasome inhibitors that is licensed for treatment of some cancers (bortezomib; Velcade®) and is effective against vaccinia virus in vitro at μM doses, has been delayed; work has therefore focused on the research-grade compound, MG-132, which is effective in vitro on vaccinia virus at a dose 10-fold higher than that used for bortezomib. The studies on variola virus have demonstrated inhibition of multiplication of three distinct isolates (Bangladesh 1974, Sierra Leone 1968, Brazil 1966). Accumulation of the late viral protein B5 was inhibited suggesting a mechanism of action similar that observed with vaccinia virus. In conclusion, MG-132 was shown to effectively halt the variola virus life-cycle at a stage prior to viral late protein expression. This class of compounds has the potential to act as potent virostatic compounds.

9.2. **Discussion:** One committee member suggested that the toxicity of proteasome inhibitors could be an issue if they were used as antiviral compounds. It was recognized that cytopathic effects were apparent with the MG-132 compound beyond one day of treatment in cell culture but that a compound such as bortezomib may be less toxic since it was effective in vitro at a lower concentration. An additional consideration is that the toxicity profile of bortezomib is well established due to its extensive use in humans as an approved treatment for multiple myeloma and mantle cell lymphoma.

10. Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention

10.1. Dr J. Kindrachuk presented results of an effort to identify new host targets for therapeutic intervention against variola virus by analysis of cell signalling pathways regulated by protein kinases (kinome). For this purpose an assay was developed based on the phosphorylation of a number of target peptides derived from proteins known to be phosphorylated by host kinases. Initial studies comparing monkeypox virus from the Congo Basin and the West African monkeypox virus demonstrated that the assay could detect significant differences in the cell kinome after infection with these two closely related viruses. The method was then applied to cell lysates from human monocytes infected with variola virus, Congo Basin monkeypox virus or as controls, lysates from uninfected cells or cells infected with gamma-irradiated variola virus. Both activated (phorbol 12-myristate 13-acetate [PMA] treated) and non-activated cells were employed for the study. Following bioinformatics analysis, preliminary information regarding the identities of host cell signalling pathways established that infected cells are differentially modulated throughout the course of variola infection compared with uninfected cells, cells treated with killed variola virus, or cells infected with monkeypox virus. Intriguingly, under all conditions tested the kinome data sets from the variola and monkeypox virus infected conditions clustered independently from one another, which suggests that the host responses to these two poxviruses differ or are regulated differently by these viruses. The results demonstrated activation and inhibition of a spectrum of protein kinases involved in a variety of protein kinase pathways, particularly the cell cycle, the Wnt pathway and insulin signalling.

10.2. **Discussion:** It was proposed that use of epithelial cells or primary cells in the future could provide further pertinent information regarding conserved host responses and/or therapeutic targets. In addition, it was proposed that modulation of other cell processes such as cytokine production would be valuable to assay in the infected cell lysates and samples have been taken for these subsequent analyses. It was also mentioned that if specific kinases could be identified as potential targets for inhibition of variola virus infection, it is important to remember that kinase inhibitors may be toxic for healthy cells.

11. Use of live variola virus to support less-reactogenic vaccine development

11.1. Dr V. Olson reported progress in investigations using live variola virus to assay for neutralizing antibodies (Nab) induced in volunteers vaccinated with less-reactogenic vaccines such as Modified Vaccinia Ankara (MVA). Variola virus neutralization is considered important for vaccine evaluation in cases where no "take" occurs. Recent collaborative work between the CDC and Harvard University on the ability of ACAM3000 MVA administered either subcutaneously, intradermally or intramuscularly to induce neutralizing antibodies against variola virus was presented. The median 50% anti-variola virus PRNT titres were similar to the 50% anti-vaccinia virus-MVA-Luc neutralization titres and higher than the 50% anti-vaccinia virus-WR-Luc titres at peak times post-vaccination. Studies comparing the subsequent anti-variola virus immune response showed that initial vaccination with ACAM3000 MVA (two doses) led to a more rapid and enhanced Nab response upon subsequent challenge with Dryvax®. The titres in Nab were highest with immunization by the intradermal route employing 10^7 PFU or by the intramuscular route employing 10^8 PFU. Further studies in a large group of volunteers are planned to evaluate the ability of MVA to induce a serological immune response in comparison to Dryvax®.

11.2. **Discussion:** Several questions were raised by Committee members, notably: whether the finding that the MVA-based neutralization assay yielded similar results to the variola virus-based assay implied that the former could substitute for the latter; what, if any, were the public health implications of the results; and whether it was not also important to titrate the cell-mediated immune response to variola antigens. In response to these remarks it was pointed out that the results obtained with variola virus neutralization assays (i) could support the use of this virus as a smallpox vaccine and (ii) indicate a comparable immune response when used as a dose-sparing, intradermal regimen (which could provide significant advantages in the event of vaccine shortages); however, the data available to date did not have the statistical power to draw firm conclusions and additional studies planned in the future may overcome such limitations. No data on the cell-mediated immune response to variola virus have been generated; these samples are not available.

12. Efficacy study of the therapeutic window of oral ST-246® in cynomolgus monkeys infected with variola virus

12.1. Dr J. Goff described research into ST-246®, an antiviral drug designed for the treatment of smallpox. Safety and efficacy of ST-246® were evaluated in cynomolgus monkeys infected with variola virus and either treated by oral gavage with 10 mg/kg per day of ST-246® or untreated. The dose of 10 mg/kg per day is considered approximately equivalent to the proposed human oral dose (400 mg/day) based on weight conversion. 18 monkeys were intravenously challenged with 10^8 PFU of the variola virus Harper strain on day 0 and administered ST-246® daily beginning on day 2 or day 4 post-infection or placebo treated for 14 additional days. Animals were monitored daily for signs of clinical illness or lesions and animals were bled at the end of the experiment to evaluate viraemia. Three of six placebo-treated animals were euthanized in moribund condition due to severe advancing variola-induced disease, while the survivors in this group displayed severe symptoms of disease prior to recovery. Group averages for maximum whole body lesion counts exceeded 1400 and viral load in the blood at maximum exceeded $5x10^6$ genome copies/ml. ST-246® treatment initiated at two or four days post-infection (one day prior to and one day after lesion onset for this model) protected the animals from mortality and significantly reduced viral load and lesion numbers relative to placebo-treated animals. The results demonstrated that daily oral administration of ST-246® at 10 mg/kg for 14 days is protective against variola virus in a non-human primate model for smallpox and suggest that similar exposure levels in humans may be effective at preventing and/or treating pathogenic orthopoxvirus infections in humans.

12.2. **Discussion:** It was noted that this study, performed with the same virus stock as a similarly designed study reported at last year's meeting but with a contrasting result, had led to the observation of clinical efficacy of the ST-246® treatment in the variola non-human primate model. Dr Goff remarked that the reason for this discrepancy could be attributed to the narrower range of weight divergence of the animals than in the previous experiment, although it was also recognized that biological variability between two small groups of animals could also be involved.

13. New generation smallpox vaccines

13.1. Dr S. Shchelkunov reported on the construction and experimental evaluation of a polyvalent DNA vaccine for smallpox. Mice were vaccinated three times at three-week intervals and by different routes (IM, IP, SC and IC) with DNA encoding several structural surface variola virus proteins (A30L, A36R, B7R, F8L and M1R). Vaccination induced high titres of anti-vaccinia neutralizing antibodies similar to those induced by live vaccinia virus immunization. The mice were completely protected from a lethal ectromelia virus challenge (10 LD_{50}) by the intraperitoneal route. In another strategy, targeted deletions were introduced into the virulence genes C3L (complement binding protein), N1L (NF-B and apoptosis inhibitory factor), A56R (haemagglutinin) and B8R (interferon gamma receptor) of the vaccinia virus vaccine strain LIVP. Viral mutants were constructed with various combinations of each deletion. Studies of the attenuation, immunogenicity and ability to protect against a challenge orthopoxvirus infection are ongoing.

13.2. **Discussion:** In response to questions, Dr S. Shchelkunov indicated that DNA vaccination had been carried out using 50µg/dose of each of the five plasmids of the polyvalent DNA vaccine under study and that the cellular immune response in the mice had been assayed for several of the DNA plasmids employed and some animals had indeed induced a high T cell immune response. Another question addressed the reason for choosing to employ variola virus genes for DNA vaccine creation and protection challenge with lethal ectromelia virus after mice immunization. In response, it was stated that since the sequences of the variola genes under investigation were more divergent from ectromelia than from other orthopoxviruses, it was thought that their efficacy in the ectromelia animal model was a good indication that they would be protective against a human smallpox infection. Concerning the second strategy presented, one question addressed the reason for choosing to delete the four vaccinia virus genes specified rather than other genes known from published studies to be virulence factors.

14. FDA efforts to facilitate the development and approval of smallpox medical countermeasures

14.1. Dr L. Borio summarized the current framework set up by the US Food and Drug Administration (FDA) to facilitate the development of smallpox medical countermeasures (MCMs) and highlighted recent progress in the regulatory review of smallpox MCMs. FDA, under its Medical Countermeasures Initiative, addresses key challenges in MCM development and regulatory review in three areas: (i) enhancing the regulatory review process for the highest priority MCMs and related technologies; (ii) advancing regulatory science for MCM development; and (iii) modernizing the legal, regulatory and policy framework to facilitate MCM development, access, and ensure an effective public health response. FDA continues to work 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, licensure or clearance. Measurable progress has been made and smallpox MCMs continue to advance in development.

14.2. In September 2012, FDA cleared a diagnostic assay for the detection of non-variola orthopoxvirus DNA to aid in the diagnosis of non-variola orthopoxvirus infection. The development of a nucleic acid based diagnostic assay for variola virus is ongoing but not yet under review by FDA. FDA's overarching goal with respect to diagnostic tests for smallpox is to balance the need for adequate validation studies to ensure the highest level of performance with the need to avoid requirements that are overly burdensome on developers.

14.3. With respect to next-generation smallpox vaccines, FDA has clarified that development and regulatory review should be under the traditional licensure pathway when possible (i.e. not via the "Animal Rule"), which includes demonstration of non-inferiority to ACAM2000® (a live smallpox vaccine licensed by FDA in 2007 that is derived from a clone of the Dryvax® smallpox vaccine, purified, and produced using cell culture technology) with supportive data from animal studies using non-variola orthopoxviruses (e.g. mouse/ectromelia virus and non-human primate/monkeypox virus). The development of IMVAMUNE® (Modified Vaccinia Ankara [MVA]), an attenuated, non-replicating smallpox vaccine, continues to progress in development. In a declared public health emergency, IMVAMUNE® may be authorized for use under an Emergency Use Authorization for smallpox vaccination in the USA to protect certain individuals at a higher risk of serious adverse events from conventional smallpox vaccines. The sponsor of LC16m8, an attenuated smallpox vaccine licensed in Japan in 1975, is also seeking the development of this vaccine in the USA.

14.4. With regard to new antiviral drugs for the treatment of smallpox, FDA has communicated to developers that their development programmes should be based on the "Animal Rule" and that principal animal model studies may rely on non-variola animal models. FDA will continue to work collaboratively to address scientific uncertainties inherent in smallpox MCM development to facilitate development and regulatory review towards approval, licensure or clearance.

14.5. **Discussion:** The Committee welcomed the increased clarity regarding the regulatory requirements for the development, regulatory review and approval of smallpox MCMs that has been provided by FDA to companies developing such MCMs. The Committee also noted that, given the totality of the available data, FDA is not stating a requirement for the use of live variola virus in animal models for the two lead antiviral drugs under advanced development. Specific pathways had not been determined for any new drugs that may subsequently reach the advanced development stage although such a prospect appeared unlikely within the next few years.

14.6. One Committee member expressed surprise at the lack of FDA approval for any variola virus nucleic acid diagnostic assay since such techniques have been established for more than 10 years. FDA noted that no such diagnostic assay has been submitted to FDA for review. Others underlined that the assays currently available display cross reactivity to non-variola orthopoxviruses (e.g. cowpox) and that it is unadvisable to take the risk of authorizing the

release of assays that could yield false-positive results and cause unwarranted alarm. One suggestion to resolve this issue was to design tests that include recognition of variola and cowpox simultaneously, such that a false-positive result for variola could potentially be recognized as cowpox. The challenge of overcoming inter-laboratory and inter-operative variability was also considered a key issue before finalization of release of any smallpox diagnostic assay. An additional hurdle in developing species-specific variola diagnostics stems from the deliberate choice of target DNA sequences within conserved essential regions to ensure they cannot be readily lost/deleted from the genome as could be the case for "non-essential" regions. In addition, the commercial viability of such kits has been a challenge; one kit with, in vitro validation against multiple strains of variola and other orthopoxviruses, was produced in 2006 but is no longer commercially available.

15. Progress towards approval of ST-246®

15.1. Dr D. Hruby, summarized progress on the development of the smallpox antiviral drug candidate tecovirimat (ST-246® or Arestvyr®) developed by SIGA Technologies, USA. The drug was discovered by a traditional high-throughput screening effort in which >350 000 compounds were screened for their ability to inhibit the replication of vaccinia virus in vitro. One of the more promising hits was matured by chemistry into the molecule called tecovirimat. Tecovirimat was found to be a highly potent, non-toxic and specific inhibitor of orthopoxvirus replication in vitro and in vivo. Tecovirimat is effective at preventing morbidity and mortality in many different animal models, from mouse to monkey, and against a number of orthopoxviruses, including variola virus. The drug is orally bioavailable with excellent pharmacokinetic parameters. The final active pharmaceutical ingredient and clinical trial material have been determined and three New Drug Application (NDA) batches have been completed and are in the midst of stability testing. The drug is currently under a contingency use Investigational New Drug application. ST-246 is not yet approved by the U.S. Food and Drug Administration. It has been accepted in to the U.S. Strategic National Stockpile under a CDC held contigency use Investigational New Drug for potential use during a declared emergency. It has an orphan drug designation in the USA for the treatment and prevention of smallpox. Most of the NDA-enabling studies have been completed with the remaining animal efficacy and human clinical trials designed and ready to launch.

15.2. **Discussion:** The Committee noted the very advanced stage of development of ST-246® as a effective drug for treatment of smallpox. In response to a question about additional animal experiments that may be required for FDA approval, it was reported that following the Advisory Panel held by FDA in December 2011, a clear blueprint for the data package required had emerged. Due to the inconsistencies of the variola non-human primate model, no pivotal data from this model will be needed. Likewise, due to the difficulties conducting controlled clinical studies in Africa, no human monkeypox efficacy data will be required. There exist several excellent animal models including the intravenous monkeypox model, the intradermal rabbitpox rabbit model and the ectromelia mouse model. It is anticipated that data from two or more of these models will be triangulated together with human data to demonstrate efficacy and establish the human dose. As these animal studies will need to be conducted under Good Laboratory Practices, some time will be required to establish the

models, validate them, and conduct the necessary studies. In response to further questions it was noted that the toxicity of ST-246® was documented as not clinically significant in animal and human studies and that the drug was secreted unmetabolized in faeces. Furthermore, it was expected that a formulation could be developed for treatment of small children.

16. Progress on the development of smallpox vaccine IMVAMUNE®

16.1. Dr L. Wegner, Bavarian Nordic, Denmark presented an update on IMVAMUNE® (MVA-BN®) – a live, highly attenuated vaccinia strain that does not replicate in human cells. IMVAMUNE® is currently being developed as a stand-alone, non-replicating, third-generation smallpox vaccine. More than 3400 subjects have been vaccinated with IMVAMUNE® in 16 completed or ongoing clinical trials. The subjects included more than 1000 persons from risk groups with contraindications for conventional smallpox vaccines (i.e. persons infected with HIV or diagnosed with atopic dermatitis). A Phase III efficacy study will begin in 2013 and a licence application for marketing authorization was submitted to Health Canada in 2011. IMVAMUNE® has been shown to be safe in both healthy individuals and those with impaired immune function. IMVAMUNE® induces a strong vaccinia-specific immune response comparable between healthy subjects and at-risk groups and is non-inferior to traditional vaccines such as Dryvax®. One or two vaccinations with IMVAMUNE® induce a long-lived immunity. IMVAMUNE® is part of the US Strategic National Stockpile of medical products, under a pre-Emergency Use Authorization for potential use in individuals of all ages with HIV infection or atopic dermatitis including children, pregnant women and nursing mothers. Registration has been filed for in Canada and Europe (European Medicines Agency) and approval from these agencies is expected within the year 2013.

16.2. **Discussion:** The Committee noted that regulatory approval of this non-replicative vaccine is in an advanced stage and that a stockpile had already been established within the USA in view of potential vaccination of individuals with relative contraindications for the traditional smallpox vaccine. It was also underlined that the major regulatory agency involved in guidance towards approval (FDA) had stated clearly the final steps that need to be achieved. In response to additional questions it was noted that the IMVAMUNE® vaccine could be made available to laboratory workers or even Collaborating Centres if approval is gained. Studies performed indicated that the stability of the vaccine was 3 years but further work was under way to develop a freeze-dried version with prolonged stability. The use of chicken embryo fibroblasts as a virus culture system was justified by the difficulty for the manufacturer to change to other promising cell culture systems at this late stage of development. To questions raised concerning potential safety issues (e.g. for HIV-infected individuals) it was stated that so far safety had been documented in people with as few as 200 lymphocytes per ml and that vaccine adverse reactions typical of killed vaccines, such as the influenza vaccine, were observed in some IMVAMUNE® vaccinees but were not observed to be severe adverse reactions. Finally, there were a few comments on the relatively high cost that might be involved in acquiring this vaccine.

17. Update on LC16m8 vaccine

17.1. Dr H. Yokote, KAKETSUKEN, Japan, provided participants with an update on the attenuated replication-competent vaccinia virus, LC16m8, which was first licensed in Japan in 1975. More recently, LC16m8 has been stockpiled in Japan since 2001 as a potential countermeasure for emergency use against bioterrorism. The LC16m8 vaccine was formerly used as a smallpox vaccine in approximately 90 000 infants and more recently has been given to over 8000 members of the armed forces in Japan and 125 volunteers in a clinical trial in the USA, without any severe adverse reactions. At a dose of 10^5 PFU, the vaccine induces a "take" and high immunogenicity. KAKETSUKEN has established an industrial plant and a manufacturing process to supply $80x10^6$ doses per year intended for emergency use. Currently, LC16m8 is licensed in Japan with 4-year shelf-life in a lyophilized formulation stored at -20°C. Extensive studies have demonstrated the ability to store the vaccine in a lyophilized formulation for longer periods of time at -20°C. In addition, the studies showed that the vaccine was stable after reconstitution for no less than 30 days at the ambient temperature.

17.2. **Discussion:** The Committee took note of the large-scale manufacturing of LC16m8, the successful establishment of lyophilization methods and the demonstration of vaccine stability for both the lyophilized formulation and reconstituted vaccine. The vaccine was considered to be effective with a single application with a bifurcated needle of 10^5 PFU because of its ability to replicate; however, boost inoculations could be recommended. In response to questions raised about contraindications, it was stated that there were no reports so far in Japan. No studies had yet been conducted in HIV-infected individuals.

18. Update on hexadecyloxypropylcidofovir (CMX001) therapeutic development for smallpox

18.1. Dr R. Lanier described the structure of CMX001 as a lipid derivative of cidofovir that has improved properties. Unlike cidofovir, CMX001 is administered orally, is not associated with dose limiting nephrotoxicity and is a potent inhibitor of multiple DNA viruses such as adenovirus, cytomegalovirus and variola virus major. CMX001 has successfully entered Phase III development for cytomegalovirus and is in Phase II for adenovirus. Recent interactions with the FDA have led to agreement that the ectromelia mouse model combined with the rabbitpox rabbit model may be used in development of CMX001 for a therapeutic indication in smallpox. Dr R. Lanier presented the progression plan based on these models developed in consultation with FDA and the US Biomedical Advanced Research and Development Authority (BARDA). CMX001 is effective in prevention of mortality in both lethal models past the halfway point in the disease. The trigger for treatment in the intradermal rabbitpox model is the first indication of secondary lesions. The proposed trigger for treatment in the mouse model is detection of ectromelia virus in saliva or blood using PCR. Recent data concerning co-administration of vaccines and cidofovir or CMX001 were described which suggest vaccine efficacy is maintained, i.e. animals survived re-challenge with lethal viral inoculums following co-administration of drug and vaccines. Manufacturing

of CMX001 has been validated at commercial scale and the drug is stable over multiple years. CMX001 is in advanced development for cytomegalovirus and adenovirus in human trials and for smallpox using the "Animal Rule" with rabbit and mouse models.

18.2. **Discussion:** The Committee noted the progress made in discussions with regulatory authorities (FDA) to establish a clear pathway for development (two non-primate animal models) that could result in approval of CMX001, although this could still be a few years away. The possibility of combining CMX001 and ST-246® in treatment of smallpox was suggested. It was noted that such a strategy, albeit attractive, would still require independent development of both drugs but would remain an option at the final stage since synergy has been demonstrated. Possible development of drug resistant viruses was not considered a major problem for CMX001 since it had been demonstrated in the vaccinia model that such mutants were highly attenuated.

19. WHO smallpox vaccines: update

19.1. Dr I. Pluut recalled that a strategic preparedness plan to deal with any smallpox outbreak had been under revision by WHO since the beginning of 2012. This revision would address procedures for case identification and confirmation and mechanisms for exchange of information. The plan would involve setting up response teams for logistic management of an outbreak to deal in particular with vaccine distribution and recommendations for its use. Dr I. Pluut recalled the WHO goal of establishing an emergency vaccine stockpile of 200 million doses that would be comprised of pledged doses as well as physical stocks held by WHO. Currently, 600 000 nominal doses (i.e., by the single syringe method) of donated smallpox vaccine as well as 300 000 doses of recently acquired ACAM2000® were in the physical stockpile. These vaccines were being tested every five years for potency, the last test having been carried out in 2010. Further advice on smallpox preparedness would be sought by WHO concerning various regulatory and practical issues related to a smallpox emergency.

19.2. **Discussion:** In response to one question concerning the type of vaccines included in the WHO stockpile it was stated that they were essentially first generation vaccines. The Committee noted that there was a discrepancy between the number of smallpox vaccine doses in the WHO stockpile reported at this meeting compared with previous meetings and requested further clarifications on the stockpile from the WHO Secretariat. The ACVVR decided that the stockpile vaccine meeting planned for early 2013 should not be under the ACVVR, reporting to the Chairman of the Committee. This should be addressed by the Ad Hoc Committee on Orthopoxvirus Infections, directly reporting to WHO. Finally, the Committee Chairman pointed out that the operational framework for dealing with any smallpox outbreak was not the remit of this Committee, which was set up to provide oversight of essential variola virus research of public health benefit.

20. Update on variola virus repositories biosafety inspection visits in 2012

20.1. Dr N. Previsani presented a brief overview of the way in which biosafety and biosecurity inspections had recently been carried out at the two WHO repositories for variola virus, CDC in Atlanta, GA, USA and VECTOR in Novosibirsk, Russian Federation. At first the WHO inspection team met with the two laboratories to revise established procedures. The written protocol followed the Laboratory Biorisk Management Standard CWA 15793:2011. Final reports of these inspections were not yet available but they would be published on the WHO web site shortly and would mention any concerns (if identified) and how they could be amended.

21. The Smallpox Laboratory Network

21.1. Dr J.-C. Piffaretti recalled that in 2010 an ACVVR subgroup produced a report on the concept of a Smallpox Laboratory Network (SLN). In 2011, the ACVVR recommended implementation of the SLN, the main objectives of the programme being: (i) to elaborate a scheme for establishing and maintaining a WHO worldwide network of laboratories capable of providing rapid and reliable screening of clinical samples suspected to contain variola virus; (ii) to integrate the SLN into a more general diagnostic laboratory network, specifically the WHO Emerging and Dangerous Pathogens Laboratory Network; (iii) to identify the molecular diagnostic techniques (PCR and real-time PCR tests) that are currently available for transfer to the SLN; (iv) to identify a mechanism to ensure quality control and proficiency assessments, including the production of positive and negative samples; (v) to identify a mechanism to ensure the production of a PCR assay for distribution to the SLN.

21.2. Unfortunately, due to several reasons including limited resources, in the current year the project did not progress as expected. At present, the candidate regional laboratories to be included in the SLN are being identified by WHO according to the criteria mentioned in the report. The SLN regional laboratories will be designated by WHO and the two WHO Collaborating Centres in the near future.

21.3. Regarding the diagnostic molecular assays for the detection of variola virus, a very limited number are still available on the market but they are intended for scientific purposes only and it is uncertain whether their production will be continued. Indeed, producing and licensing such diagnostic tests appears to have limited commercial viability. An attractive possibility would be to take advantage of two diagnostic assays established by the WHO Collaborating Centres, one in the process of being licensed by FDA and the other already licensed in the Russian Federation. In order to accelerate the establishment of a diagnostic assay for the SLN regional laboratories, the creation of a small technical group was suggested, which would comprise representatives of the WHO Collaborating Centres and one or two experts. This group would have the task to evaluate the assays presently in use and to select the more adequate test for the regional laboratories. It was pointed out that resources are mainly needed to implement proficiency assays, to provide adequate training and for inspections. Without appropriate funding, it will not be possible to establish the SLN.

21.4. **Discussion:** The issue of the establishment of the SLN, in particular the designation of the regional laboratories was reviewed. WHO has informed the Regional Offices of the procedure, and is consulting with them about the identification of candidate laboratories and the designation of the network.

21.5. Considering that, in theory, samples from suspected smallpox cases may be sent quickly to one of the two WHO Collaborating Centres, the issue of the real usefulness of the SLN was also raised. In response it was noted that this may not be true everywhere because of the difficulty of transportation of highly infectious samples, for instance in Africa: a more local laboratory would provide faster results. Other items addressed during the discussion were the utility of electron microscopy as a screening tool, the safety rules for sample transportation and the role of the SLN in the WHO Emerging and Dangerous Pathogens Laboratory Network.

22. General discussion

22.1. A general discussion followed the formal presentations and dealt with the advances reported in drugs, vaccines, animal models and diagnostics for smallpox. The progress made in demonstrating efficacy, safety and achieving regulatory approval for the use of two drugs (ST-246® and CMX001) with different mechanisms of action was underlined. It was noted that no additional research using live variola virus in animal studies was being required by one regulatory agency (FDA). This however was not considered to apply to any new drugs that may subsequently reach the drug development stage. There appeared to be a requirement for the precise measurement of the in vitro efficacy of one of the drugs (EC_{50} for CMX001) against variola virus, an experimental value still unavailable. There was considerable debate as to whether research should be pursued into more than the two drug candidates in the most advanced stage of development. On the one hand, it was pointed out that not all drugs that undergo the ultimate Phase III trials necessary for approval ever actually gain licensure even though the success rate of antimicrobial agents is relatively good (70%). On the other hand, it was recognized that if one of the two leading drugs failed to gain licensure then an additional one may be thought necessary and this would require a number of years given the very preliminary state of research into other existing candidates. The discovery of anti-variola activity in a currently marketed drug would of course streamline the process considerably since safety data would already be available. It was concluded that research into additional anti-variola drugs should not continue once licensure of two drugs has been achieved.

22.2. The availability of licensed smallpox vaccines and progress towards licensure of more highly attenuated vaccines was discussed. The attenuated cell cultured vaccine LC16m8 had already gained licensure approval in Japan since 1975 and a cloned cell cultured vaccine derived from the traditional smallpox vaccine Dryvax® (ACAM2000®) was approved in 2007 in the USA. Both of these vaccines are recommended in case of an emergency situation for use in the entire population with very few contraindications. An emergency use of the highly attenuated IMVAMUNE® vaccine would also be possible for a subgroup of individuals with relative contraindications to live smallpox vaccine in the USA. Licensure of both

IMVAMUNE® and LC16m8 are being sought in countries other than those mentioned. The Committee recognized that to finalize regulatory approval of attenuated smallpox vaccines, limited in vitro work (assay of neutralizing antibodies) with live variola virus would complete assessment in volunteers. Questions remained about the WHO stockpile, its size, maintenance and lifetime.

22.3. There was general agreement in the Committee that no further research on live variola virus was necessary in the area of nucleic acid or protein diagnostics as this could be carried out on viral DNA or previously inactivated samples. There was also some disappointment about the lack of readily available diagnostic kits for smallpox, with recognition that there were legitimate scientific hurdles and funding difficulties because of limited commercial interest.

23. Conclusions

Drugs

23.1. The Committee received updates on the two anti-smallpox drugs that are closest to licensure. This included very positive data on efficacy, safety, stability and large-scale manufacturing capacity. One regulatory agency (FDA) stated that no further use of live variola virus was necessary for these two products in their path to licensure. Instead, specific efficacy studies with orthopoxvirus infections in animal models were requested (e.g. ectromelia virus in mice and rabbitpox virus in rabbits). The Committee welcomed this clarity in guidance towards licensure. The companies are now in the process of undertaking this work, which is estimated to take not less than one year.

23.2. The Committee also heard reports on other compounds that were all in a very early stage of evaluation as antivirals for variola virus. The Committee noted that initial evaluation of these compounds should utilize orthopoxviruses excluding variola virus and only the most promising compounds should be considered thereafter for work with live variola virus. It was also noted that drugs that were licensed already for other purposes and which showed efficacy against variola virus faced fewer developmental challenges than other new compounds.

Vaccines

23.3. The Committee noted the very positive progress towards gaining regulatory approval for one new generation smallpox vaccine (IMVAMUNE®). This had been submitted for licensure in Canada and the European Union and a decision was expected in 2013. In addition, the Committee heard an update on the manufacturing of another attenuated smallpox vaccine (LC16m8), which has been licensed in Japan since 1975 and for which licensure is sought in other countries. Both IMVAMUNE® and LC16m8 were being manufactured in large quantities and they showed promising stability. LC16m8 is currently manufactured in a lyophilized form and its stability is being evaluated further. For IMVAMUNE®, a lyophilized version is under development and evaluation. Some additional work with variola virus is planned to evaluate the induction of neutralizing antibodies in volunteers vaccinated with these vaccines.

Diagnostics

23.4. The Committee noted that currently no nucleic acid based detection assay for smallpox was licensed. However, a review of this topic conducted in 2010 reported that two diagnostic kits for orthopoxviruses had been developed and other assays were reviewed. One of these assays, a real-time PCR assay, best studied against a broad panel of orthopoxviruses, is no longer commercially available. The other assay, a lateral flow assay, has recently been evaluated against a limited number of monkeypox virus specimens.[1] Currently, a variola virus-specific diagnostic test that would distinguish variola virus from other orthopoxviruses (including recently described cowpox viruses) was being refined based upon the existing tests. Research using live variola virus was not considered necessary for this development although genomic variola virus material would be required. The Committee concluded that

[1] Townsend MB et al. Evaluation of the Tetracore Orthopox BioThreat(®) antigen detection assay using laboratory grown orthopoxviruses and rash illness clinical specimens. *Journal of Virological Methods*, 2013, 187:37–42.

there was an urgent need for the availability of at least one nucleic acid based diagnostic assay enabling sensitive, specific and validated diagnosis of smallpox.

The WHO smallpox vaccine stockpile

23.5. The Committee noted that individual nations have pledged a total of 31 million doses to WHO towards the goal of the virtual stockpile of 200 million doses set by WHO. The Committee also noted that currently WHO held 600 000 nominal doses (i.e., by the single syringe method) of first generation smallpox vaccines, and that the potency of this stockpile is evaluated regularly. There were also 300 000 doses of ACAM2000® purchased for this stockpile in Switzerland.

The inspection of WHO Collaborating Centres

23.6. The Committee heard that both Collaborating Centres had been inspected during 2012 and that final reports were being drafted and would be published on the WHO web site once finalized. The inspection teams and the Collaborating Centres were thanked for their hard work and cooperation during these inspections.

The Smallpox Laboratory Network

23.7. The Committee heard that limited progress had been made due to a variety of logistic issues. WHO informed the Committee that the laboratories would be chosen from existing diagnostic laboratories with expertise in dangerous pathogens.

Annex 1. Summaries of the presentations

Update on research proposals submitted to WHO 2011/2012

Scientific subcommittee members: **Clarissa Damaso, Grant McFadden, Andreas Nitsche, Jean-Claude Piffaretti, Tony Robinson, Li Ruan, Oyewale Tomori**.

Coordinator: **Robert Drillien**

November 18 2011 (review date):

Proposal submitted by CDC, Atlanta

- Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention.
  - Five of seven members recommended approval.

December 21 2011 (review date):

Proposal submitted by VECTOR, Kolstovo

- Discovery of new antiviral for smallpox treatment and prevention.
  - Approval recommended.

February 9 2012 (review date):

Proposals submitted by CDC, Atlanta (six reviews submitted)

- Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus.
  - Approval recommended.

- Use of live variola virus to evaluate antivirals against variola.
  - Approval recommended.

- Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of "third" generation vaccines.
  - Approval recommended.

- Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support.
  - Approval recommended for the purpose of improving the current DNA and protein based diagnostic methods. Three of six reviewers did not recommend approval of the part of the proposal involving sequencing of additional variola virus genomes

March 12 2012 (review date):

Proposal submitted by CDC, Atlanta

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

Proposals submitted by VECTOR, Kolstovo

- Assessment of the neutralizing activity of vaccinee blood sera using live variola virus.
    - Six of seven members recommended approval.

- Development of therapeutic anti-smallpox antibodies.
    - Approval recommended.

Report on the variola collection at the WHO Collaborating Centre Repository in SRC VB VECTOR

A.N. Sergeev, L.E. Bulychev

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, as well as 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 and emergency situations.

Currently, the VARV collection comprises 120 strains, originating from Europe, Asia, Africa, South America and 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 is 696.

The VARV stocks are stored in polypropylene cryovials. In 2012, the Ind-3a variola virus strain was grown in Vero cells and used to assess antiviral properties of chemically synthesized compounds and therapeutic antibodies.

Research using live variola virus will be continued in 2012–2013 to:

- discover new antiviral chemically synthesized compounds for treatment and prevention of smallpox;
- develop smallpox therapeutic antibodies;
- assess variola virus neutralizing activity of sera from those vaccinated against smallpox;
- develop animal models to study the efficacy of therapeutic and preventive preparations against smallpox (after consultation with the WHO).

The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention, Atlanta, GA, USA: 2012 report on the variola collection

Victoria Olson, Kevin Karem, Paul Hudson, Zachary Braden, Scott Smith, Cody Clemmons, Christine Hughes, Inger Damon

Poxvirus Program, Centers for Disease Control and Prevention, Atlanta, GA, USA

The WHO Collaborating Center for Poxviruses 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 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 2011 and 2012. A working stock of purified variola virus Harper has been prepared and stored as single use aliquots for use in animal studies in 2012. 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: development and use of a murine model for evaluation of smallpox medical countermeasures; finalization of 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.

Between November 2011 and September 2012 there were removals of variola virus or samples from prior studies from the repository for WHO-sanctioned protocols. During December 2011, original scabs or homogenates were processed (not propagated) for sequencing under the WHO-approved protocol. The laboratory and research activities were evaluated by the WHO inspection team from May 7–11, 2012. The laboratory space was in active use from November 2011 through late March 2012; the laboratory underwent decontamination prior to preventive maintenance in April 2012. After completion of the WHO inspection in May 2012, the laboratory once more became operational in mid-May 2012.

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

Victoria Olson, Scott K. Smith, Zachary Braden, Paul Hudson, Christine Hughes, Whitni Davidson, Kevin Karem, Inger Damon

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Additional external collaborators: R. Lindsey Baden, MD, Harvard University

This protocol was last renewed (April 2012) and is valid through to December 2012.

In the absence of an animal model utilizing variola virus that mimics human smallpox, variola virus neutralization in vitro remains one of the only measures of vaccine efficacy. Differences in antigenic makeup suggest that neutralization may differ between target viruses using sera from vaccinia virus vaccinees (heterologous target versus homologous target). 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 end-point for the evaluation of vaccines. However, vaccinee sera'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.[2]

The development of new vaccines has included significant focus on the use of Modified Vaccinia Ankara (MVA) virus-derived vaccines. MVA and other attenuated vaccine strains, such as Lc16m8, 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.

Results from collaboration with St. Louis University (DMID 02-017), comparing variola virus neutralizing responses of various smallpox vaccines, and the comparison of variola virus and vaccinia virus neutralizing responses have been published. A second collaboration with researchers at Harvard University (DMID 05-0010) continues. In 2010 and 2011, the results on the ability of the subcutaneous and intradermally delivered vaccines (MVA) to neutralize variola virus MV were presented. The median 50% anti-variola virus PRNT titres were similar to the 50% anti-vaccinia virus-MVA-Luc neutralization titres and higher than the 50% anti-vaccinia virus-WR-Luc titres at peak times post-vaccination. Studies comparing the subsequent anti-variola virus immune response, and clinical response, to a Dryvax® "challenge", and evaluation of the kinetics of the anamnestic response are in final stages of analysis.

[2] Smith et al. The formation and function of extracellular enveloped vaccinia virus. *Journal of General Virology*, 2002, 83:2915–2931

Discovery of new antivirals for smallpox treatment and prevention/ Development of therapeutic anti-smallpox antibodies/ Assessment of the neutralizing activity of vaccine blood sera using live variola virus

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In 2010–2011, it was demonstrated by SRC VB VECTOR that the compound NIOCH-14 is comparable to a compound that is part of ST-246® in terms of antiviral activity as this was shown in a cell culture study involving the use of variola virus and in experiments to study the suppression of monkeypox virus replication in the lungs of mice. It was also shown that infection development following intranasal inoculations of marmots (*Marmota bobac*) is associated with pronounced clinical signs of the disease, which makes it possible to use marmots as an animal model to evaluate the effectiveness of therapeutic and preventive products.

In 2012, therapeutic and prophylactic activity of NIOCH-14 and that of the compound that is part of ST-246® was studied in an experimental monkeypox virus infection in marmots infected intranasally with the monkeypox virus strain, V79-1-005, at a dose of 30 ID_{50}. NIOCH-14 and the compound that is part of ST-246®, administered orally at a dose of 40 mg/kg, completely suppressed the manifestation of clinical signs of disease in marmots. All the animals that received treatment revealed antibodies that neutralized live monkeypox virus.

New experimental data on monkeypox virus replication patterns in organs and tissues of intranasally infected marmots and mice were obtained. It was demonstrated that mortality in marmots intranasally infected with monkeypox virus does not exceed 50% and hardly depends on the dose of infection, in contrast to the ID_{50} value, which has a strong "dose–effect" relation, and at doses of 10 ID_{50} and higher it causes 100% infection of the animals, as assessed by the manifestation of clinical symptoms of the disease.

Antiviral activity of four new compounds against the Ind-3a variola virus strain was studied in vitro. A new compound has been identified that is considered to have a potential as a promising drug against orthopoxviruses.

Two purified recombinant fully human smallpox antibodies have been produced at a concentration of 1 mg/ml. In a neutralization test using the Ind-3a variola virus strain in vitro, these antibodies were found not have reliable neutralizing activity.

The work to assess smallpox antibody levels in the sera of individuals vaccinated with new variants of smallpox vaccines is scheduled to start in the second half of 2012 and continue into 2013. This effort will involve the use of variola virus neutralization in experiments in vitro.

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

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1) Purpose/ Public Health Relevance

A number of animal models of systemic orthopoxvirus disease have been developed to evaluate various safer smallpox vaccines or therapeutics. These models have short disease incubation periods, which do not resemble what is seen in human smallpox. As a result, these systems 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, other primates 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 ($1x10^8$–$1x10^9$ 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, inbred mouse strains are relatively difficult to infect, and obtain symptomatic illness, with monkeypox virus. A recent study surveying a large panel of inbred mouse strains has identified a strain (CAST/EiJ) which is highly susceptible to infection with monkeypox virus.[3] 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 immunological reagents, and ease of animal handling – makes it of great interest to determine if they are susceptible to disease.

2) Aims

- Identify if CAST/EiJ mice are susceptible to variola virus infection.
- Evaluate the disease pathogenesis of variola virus within the CAST/EiJ mouse.
- Determine if the morbidity and mortality of variola virus infection in CAST/EiJ mice is dose dependent.

3) Results

CAST/EiJ female mice, 8–9 weeks old, were obtained from Jackson Laboratories (Bar Harbor, ME).Mice were cohoused in groups of 2–3 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

[3] Americo J L, Moss B, Earl PL. Identification of wild-derived inbred mouse strains highly susceptible to monkeypox virus infection for use as small animal models. *Journal of Virology*, 84(16):8172–8180).

to mouse chow all animals received treats as appetence monitors, as well as a plastic nests and other enrichment materials.

Groups of five (5) mice were inoculated intranasally on day 0. The inoculum was diluted to 10:l (5:l per nostril) in phosphate-buffered saline using sucrose-cushion-purified Harper strain of variola virus to achieve 5 different viral doses ($5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$ or $5x10^6$ plaque-forming units). Three (3) mice were mock infected using the equivalent volume of gamma-irradiated $5x10^5$ virus, two were additionally mock infected with diluent. Daily observations of the animal's food consumption, activity level, weight, rash and general appearance were recorded. Clinical criteria were used to assess for euthanasia criteria. Under anaesthesia with 3–5% of isoflurane gas; oral, ocular, and anal swabs; temperatures and a complete skin exam were (under)taken three times a week. At day 21 post-infection the animals were humanely euthanized for necropsy. Animals exhibited clinical signs (nasal and oral oedema, weight loss) and symptoms (reduced grooming, reduced activity) of illness. Signs and symptoms were more profound in animals challenged with higher viral inocula. No animals succumbed to illness. The antibody production and viraemia of the mice will be measured by real-time PCR, viral isolation in BSC-40 cell culture, plaque reduction neutralization test, western blots and ELISA. After final analysis of data, considerations will be made as to re-evaluate with younger CAST/EiJ mice, 5–7 weeks old, reportedly more susceptible to symptomatic, and severe, orthopoxvirus illness.

Use of live variola virus to evaluate antiviral agents against smallpox

Victoria Olson, Scott Smith, Kevin Karem, Inger Damon

Poxvirus Program, Centers for Disease Control and Prevention, Atlanta, GA, USA

Additional external collaborators: Michele Barry, PhD, University of Alberta

This protocol was last renewed (April 2012) and is valid through to December 2012.

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. 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 Teale et al[4] demonstrated that compounds which blocked proteasome function were able to prevent formation of viral replication factories, indicating action at a unique stage of the viral life-cycle. Efforts had planned to focus on evaluating Velcade®, and related compounds for in vitro activity against variola virus. The process has been initiated to attain a Materials Transfer Agreement with the company that manufactures Velcade®. The application has passed initial stages of company review. It is likely that another extension will be needed to acquire the compound and conduct efficacy testing. Work with a research-grade compound, MG-132, which was demonstrated to inhibit formation of viral replication factories has been initiated.

This presentation will briefly update work on the evaluation of proteasome inhibitory compounds for activity against variola virus, and work to demonstrate the EC_{50} of CMX001 (the orally available derivative of cidofovir) against strains of variola virus with various non-synonymous coding changes in the viral drug target, the DNA polymerase.

[4] Teale A et al. Orthopoxviruses require a functional ubiquitin-proteasome system for productive replication. *Journal of Virology*, 2009, 83(5):2099–2108.

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

Victoria Olson, Paul Hudson, Kevin Karem, Inger Damon

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Additional external collaborators: Jason Kindrachuk, Victoria Jensen, Peter Jahrling, National Institutes of Health

This protocol was approved November 29, 2011.

With the cessation of routine vaccinia virus vaccination following the declaration of global smallpox eradication in 1980 a significant portion of the global population has been left vulnerable to variola virus, the aetiological agent of smallpox. Thus, concerns have been raised regarding the potential impact of an outbreak of variola virus in an increasingly vulnerable population. Indeed, the increasing incidence of the closely related orthopoxvirus family member, monkeypox virus, lends further credence to the increasing vulnerability of today's society and highlights the importance for the design and development of novel antiviral therapeutic strategies for potential outbreak response. However, there is a paucity of information regarding the molecular mechanisms through which variola virus is able to modulate or subvert the host immune response; there are differences between the immunomodulatory proteins expressed by monkeypox and variola viruses. Thus, this project focuses specifically on the identification of novel host targets for therapeutic intervention through the functional global delineation of the host signalling pathways targeted by variola virus.

Many host responses are regulated independently of changes in transcription or translation and are instead regulated through post-translational modifications. Thus, global investigations of the activation state of host kinases, i.e. the kinome, through high-throughput peptide kinome arrays provide a functional mechanism for identifying host cell signal transduction pathways altered during disease pathogenesis. Several studies have demonstrated that pharmacological targeting of cellular processes may inhibit variola virus multiplication and enable prophylaxis. This research proposal utilizes global functional kinome screens to identify further host therapeutic targets and also provide novel information regarding the molecular mechanisms of variola virus disease pathogenesis. Greater understanding of how variola virus modulates the cellular environment will be critical for identification of which other orthopoxvirus infection provides the best surrogate system. The information will also assist in better characterization of animal models of systemic orthopoxvirus disease and their relatedness to smallpox disease progression – in particular, enhancement and/or modification of the non-human primate model system of smallpox disease.

This presentation will briefly update work on the functional mapping of the global activation state of host cell signalling pathways following variola virus infection in two different monocyte cell lines; comparing the response in both activated and non-activated cells. We will also discuss identification of any host kinase targets with promise for potential therapeutic intervention. Finally, preliminary comparison of host cell signalling response to variola virus infection with those of other orthopoxvirus family members, such as monkeypox virus, will be described.

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

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Additional external collaborators: R. Lindsey Baden, MD, Harvard University

This protocol was last renewed (April 2012) and is valid through to December 2012.

In the absence of an animal model utilizing variola virus that mimics human smallpox, variola virus neutralization in vitro remains one of the only measures of vaccine efficacy. Differences in antigenic makeup suggest that neutralization may differ between target viruses using sera from vaccinia virus vaccinees (heterologous target versus homologous target). 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 end-point for the evaluation of vaccines. However, a vaccinee sera'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.[2]

The development of new vaccines has included significant focus on the use of Modified Vaccinia Ankara (MVA) virus-derived vaccines. MVA and other attenuated vaccine strains, such as Lc16m8, 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.

Results from collaboration with St. Louis University (DMID 02-017), comparing variola virus neutralizing responses of various smallpox vaccines, and the comparison of variola virus and vaccinia virus neutralizing responses have been published. A second collaboration with researchers at Harvard University (DMID 05-0010) continues. In 2010 and 2011, the results on the ability of the subcutaneous, and intradermally delivered vaccines (MVA) to neutralize variola virus MV, were presented. The median 50% anti-variola virus PRNT titres were similar to the 50% anti-vaccinia virus-MVA-Luc neutralization titres and higher than the 50% anti-vaccinia virus-WR-Luc titres at peak times post-vaccination. Studies comparing the subsequent anti-variola virus immune response, and clinical response, to a Dryvax® "challenge", and evaluation of the kinetics of the anamnestic response are in final stages of analysis.

ST-246® (tecovirimat) protects non-human primates from major morbidity and mortality in an intravenous model of variola when treatment is initiated pre- or post-lesion onset

Arthur Goff[1], Eric Mucker[1], Joshua Shamblin[1], Carly Wlazlowski[1] Inger Damon[2], Allison Williams[2], Victoria Olson[2], Kevin Karem[2], Christine Hughes[2], Paul Hudson[2], Cody Clemmons[2], Doug Grosenbach[3], Dennis Hruby[3]

[1]United States Army Medical Research Institute of Infectious Diseases, [2]Centers for Disease Control and Prevention, [3]SIGA Technologies, Inc., USA

ST-246® (United States Adopted Names Designation: tecovirimat) is a recently identified small molecule tricyclononene compound (molecular weight, 376) that was discovered through a deliberate effort to develop orally available antiviral drugs for use in biodefence. It is a novel orthopoxvirus egress inhibitor that is being developed as a therapeutic to treat pathogenic orthopoxvirus infections. A randomized, double-blind, placebo-controlled study was conducted in a non-human primate model of the variola virus disease course to evaluate therapeutic efficacy of ST-246® at the anticipated efficacious dose in humans.

Eighteen cynomolgus macaques (9 male, 9 female) were infected with $1x10^8$ PFU of variola virus (strain Harper) by intravenous injection. Placebo (n=6) or ST-246® was administered once per day by oral gavage at 10 mg/kg starting treatment at 2 (n=6) or 4 (n=6) days post-infection (pi) for 14 days. Animals were bled to evaluate viraemia and monitored for signs of clinical illness and lesions daily.

Three of six placebo-treated animals were euthanized in moribund condition due to severe advancing variola-induced disease, while the survivors in this group displayed severe symptoms of disease prior to recovery. Group averages for maximum whole body lesion counts exceeded 1400 and viral load in the blood at maximum exceeded $5x10^6$ genome copies/ml. ST-246® treatment intiated at 2 or 4 days pi (one day prior to and one day after lesion onset for this model) provided 100% protection from mortality and significantly reduced viral load and lesion numbers relative to placebo-treated animals.

These results demonstrate that once daily oral administration of ST-246® at 10 mg/kg for 14 days is effective against variola virus in a non-human primate model for human smallpox and suggest that similar exposure levels in humans may be effective at preventing and/or treating pathogenic orthopoxvirus infections in man.

New generation smallpox vaccines

S.N. Shchelkunov, R.A. Maksyutov, I.V. Kolosova, S.N. Yakubitskiy, A.N. Sergeev

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

The goal of this work was to develop new safe vaccine against variola virus (VARV). We have constructed polyvalent DNA vaccine against smallpox based on variola virus genes MIR, A30L and F8L of intracellular virion surface membrane proteins as well as A36R and B7R of the extracellular virus envelope proteins under control of cytomegalovirus or Rous sarcoma virus promoters. Such vaccine induced production of a high titres of vaccinia virus neutralizing antibodies in mice similar to those elicited by the live vaccinia virus immunization. Mice vaccinated by created DNA vaccine were completely protected against a lethal (10 LD_{50}) challenge with highly pathogenic ectromelia virus.

Another direction of our investigation is development of safe candidate live vaccine against smallpox based on vaccinia virus (VACV) strain LIVP, which was used earlier for smallpox vaccination in the Russian Federation, with targeted deletions of several genes encoding virulence factors. Comparative analyses of attenuation and immunogenicity of the created variants of candidate live vaccine were done.

Preclinical studies are under way with following clinical investigations of created variants of smallpox vaccines.

FDA efforts to facilitate the development and approval of smallpox medical countermeasures

Luciana Borio

US Food and Drug Administration, USA

The US FDA 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 approval[5] – as well facilitating timely access to MCMs in the event of a public health emergency.

The US Government is supporting the development of smallpox MCMs, including drugs, vaccines and diagnostic tests. This presentation highlights the regulatory progress made since this was last presented to the ACVVR in October 2011.

FDA's regulatory mechanisms (e.g. approval or Emergency Use Authorization) for ensuring access to MCMs during public health emergencies are based on risk assessments anchored in scientific evidence. Smallpox MCMs present unique and complex regulatory challenges as the scientific evidence upon which regulatory decisions are based is difficult to obtain owing to the fact that there is no smallpox disease in the world and because animal models that adequately represent human disease are not available.

In August 2010, FDA launched its Medical Countermeasures Initiative (MCMi) to address key challenges in MCM development and regulatory assessment. MCMi focuses on three areas: (i) enhancing the regulatory review process for the highest priority MCMs and related technologies; (ii) advancing regulatory science for MCM development; and (iii) modernizing the legal, regulatory and policy framework to facilitate MCM development, access, and ensure an effective public health response.

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. Measurable progress has been made and smallpox MCMs continue to advance in development.

[5] 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.

Progress towards approval of ST-246®

Dennis Hruby

SIGA Technologies, Inc., USA

SIGA's smallpox antiviral drug candidate, which is called tecovirimat (ST-246®), was discovered by a traditional high-throughput screening effort in which >350 000 compounds were screened for their ability to inhibit the replication of vaccinia virus in vitro. One of the more promising hits was matured by hit-to-lead chemistry into the molecule we call tecovirimat. To summarize its development, tecovirimat is a highly potent, non-toxic and specific inhibitor of orthopoxvirus replication in vitro and in vivo. Tecovirimat is effective at preventing morbidity and mortality in many different animal models, from mouse to monkey, against a number of orthopoxviruses, including variola virus. The drug is orally bioavailable with excellent pharmacokinetic (PK) parameters. We have identified final active pharmaceutical ingredient (API) and clinical trial material and our three New Drug Application (NDA) registration batches have been completed and are in the midst of stability testing. The drug has an open Investigational New Drug Application (IND) with Fast-Track status. It has an approved orphan drug designation in the USA for the treatment and prevention of smallpox. Most of the NDA-enabling studies have been completed, with the remaining animal efficacy and human clinical trials designed and ready to launch, pending discussions with regulatory agencies about the essential data packages required to support approval.

Progress on the development of smallpox vaccine IMVAMUNE®

Lars Staal Wegner

Bavarian Nordic A/S, Denmark

IMVAMUNE® (MVA-BN®) is a live, highly attenuated vaccinia strain vaccine that does not replicate in human cells and is being developed as a stand-alone smallpox vaccine. IMVAMUNE® has been tested in more than 3400 subjects including more than 1000 subjects from risk groups with contraindications for conventional smallpox vaccines, i.e. HIV-infected and atopic dermatitis patients.

IMVAMUNE® has in 16 completed or ongoing clinical trials demonstrated to be safe in healthy individuals as well as in populations with impaired immune function. IMVAMUNE® induces a rapid and strong vaccinia-specific immune response comparable between healthy subjects and at-risk groups, and is non-inferior to traditional vaccines like Dryvax®. Furthermore, one or two vaccinations with IMVAMUNE® induce a long-lived immunity. This confirms that IMVAMUNE® is a suitable candidate for use in the general adult population including those with contraindications to conventional smallpox vaccines.

IMVAMUNE is currently part of the United States Strategic National Stockpile of medical products and other countries have also started to implement IMVAMUNE® in their preparedness. In 2012, the US Government expanded the population that is eligible to receive IMVAMUNE® during an emergency. In the event of a public health emergency involving smallpox, the government may now authorize the use of IMVAMUNE® to protect individuals of all ages with HIV infection or atopic dermatitis (AD). Children, pregnant women, and nursing mothers with HIV or AD may receive IMVAMUNE®.

IMVAMUNE® has been filed for registration in both Canada and Europe (European Medicines Agency; EMA) and is expected to be licensed in 2013, under the trade names IMVAMUNE® and IMVANEX® (EMA).

Update on the attenuated smallpox vaccine LC16m8

Hiroyuki Yokote

The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN), Japan

In the 1970s, an attenuated replication-competent vaccinia virus, LC16m8, was developed from the Lister strain by serial passaging in primary rabbit kidney cells. LC16m8 has demonstrated low neurovirulence and a good protective efficacy in animal models. The LC16m8 vaccine has been given to approximately 90 000 infants and, recently, to over 8000 members of the armed forces without any severe adverse effects. Based on the clinical data obtained during development, the LC16m8 vaccine was licensed in Japan in 1975. Subsequently, however, the vaccination with smallpox vaccine was ceased in 1976. Recently, the manufacturing of the LC16m8 vaccine was resumed and the vaccine has been stockpiled against potential bioterrorism with smallpox virus in Japan since 2001.

Currently, the LC16m8 vaccine is intended for emergency use. KAKETSUKEN has 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 in an emergency. In this regard, I will introduce some data from the results of actual production.

In addition, in light of a vaccine for stockpile, it is desirable that the LC16m8 vaccine maintains high stability as long as possible for a long storage. With the results obtained from long-term stability studies as well as a study for stability of reconstituted vaccine, I will demonstrate the stability profile of the LC16m8 vaccine.

Once you see the data, I'm sure you will find how the LC16m8 vaccine is suitable as a stockpiled vaccine and to be used at an outbreak event.

Update on CMX001 therapeutic development for smallpox

Randall Lanier

Chimerix Inc., Durham NC USA

CMX001 has activity against orthopoxviruses, including the causal agent of smallpox, *Variola major*. The observed broad spectrum activity of CMX001 against various species of orthopoxviruses was anticipated based on the viral polymerase-mediated mechanism of action in conjunction with the high homology among viral polymerases within the *Orthopoxvirus* genus. Resistance was slow to develop in vitro and was associated with decreased viral fitness in vitro and in vivo.

Chimerix is developing CMX001 for the treatment of smallpox under the "Animal Rule" and for other clinical indications (prevention of cytomegalovirus and pre-emption of adenovirus disease in immunosuppressed patients) using human clinical trial-based pathways to approval. It is in Phase III development with over 800 patients evaluated for multiple viral disease indications, a safety assessment package complete with chronic toxicology studies and a validated commercial scale manufacturing process. Recent advances in the development of CMX001 include: (i) successful completion of a Phase II clinical study and End of Phase II Meeting with FDA for prevention of cytomegalovirus reactivation post- haematopoietic stem cell transplantation; (ii) an increased understanding of the interaction with poxvirus vaccines; (iii) successful transfer of the lesional rabbit model to an independent contract research organization; (iv) agreement with FDA on the ectromelia mouse model as a second species for assessment of the efficacy of CMX001 for the treatment of smallpox; and (v) conceptual agreement with FDA on the path forward for development of CMX001 as a treatment for smallpox and scaling from animal models to a human dose.

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

Smallpox vaccine stockpile

Isis Pluut

Smallpox WHO Secretariat

The WHO Emergency vaccine stockpile was created based on WHA 33.4 with the aim of having 200 million doses ready available to respond to a smallpox event. It consists of a physical stockpile (of 600 000 doses first generation vaccines and 300 000 doses second generation vaccines) based in Switzerland and a virtual part pledged by several countries. The pledged part to date consists of 31.01 million doses.

The stock in Switzerland is available for first response. The potency of the vaccines was last tested in 2010 and will be tested in 2013. Depending on the potency, the actual number of vaccines available can be calculated.

The smallpox secretariat collaborates closely with: 1) WHO's legal office to finalize the terms and conditions that accompany the donated vaccines; WHO's Immunization, Vaccine and Biological group for the regulatory aspects; and 3) the logistics department to ensure that in case of a smallpox event all vaccines will be send as fast as possible to ensure a rapid response. The secretariat participates in the Global Health Security Initiative Medical Countermeasures smallpox vaccine pilot project working group. As a result of this collaboration, among others, standard operation procedures have been developed for both donating and requesting countries, the operational framework has been updated and a smallpox vaccine request has been prepared. In 2013 an information package will be prepared on vaccine safety to assist countries in a risk–benefit analysis to make informed decisions and a regulatory package will be developed with information on the vaccine safety, efficacy and regulatory status to facilitate the rapid authorizing/license for use.

In 2013, WHO aims to have a meeting to discuss the composition and size of the smallpox vaccine stockpile. Regulatory discussions will continue regarding the options for emergency use of vaccines and, with national regulatory authorities, the options for receiving vaccines that are not licensed and/or non-prequalified.

Variola virus repositories biosafety inspection visits 2012

Nicoletta Previsani

World Health Organization

There are currently two WHO Collaborating Centre repositories that work with smallpox virus; one is situated at the Centers for Disease Control and Prevention (CDC) in Atlanta, USA and the other at the State Research Center of Virology and Biotechnology (VECTOR) in Novosibirsk, Russian Federation. World Health Assembly resolution WHA60.1 (2007) mandates WHO to inspect these two centres every two years to ensure that "the conditions of storage of the virus, and that the research done in the laboratories meet the highest requirements of biosafety and biosecurity". In addition, WHA60.1 requests that inspection-mission reports be made available for public information after appropriate scientific and security redaction.

In agreement with CDC and VECTOR the inspection protocol used in 2009 was used again for the inspections of 2012. The protocol is based on the publication of the international Laboratory Biorisk Management Standard, which is a consensus Workshop Agreement registered with the European Committee for Standardization (CEN) CWA 15793:2011. The protocol addresses 16 elements relating to laboratory biorisk management.

A meeting with WHO and representatives of CDC and VECTOR took place in Oslo, Norway, between 31 January and 2 February 2012 to review the process for the biosafety inspection visits of the two smallpox repositories. During that meeting agreement was reached on a variety of issues, including the inspection team composition; the draft agenda for the visits; the desire to inspect the facilities when they were accessible to all team members and not in active use to permit evaluation of the laboratory facilities; and how the findings and report would be presented (i.e. a close-out session on the last day of the visit, followed by a written narrative report). The role of representatives from the repository not being inspected was identified by the WHO Office of the Legal Counsel to be the one of observers. Observers were able to attend interviews and site tours during the visit, but not discussions regarding findings and key observations, nor were they present at the close-out meeting.
As in 2009, the inspection process consisted of discussions and interviews with key stakeholders, record checks, programme verification and site inspections. Key findings (areas of nonconformity to CWA 15793:2011) and observations (areas that could benefit from improvement and may become a finding if not addressed before the next inspection visit) were presented for each element on the last day of the visit.

The reports of the 2012 visits are currently being finalized. In response to the inspection visit and the final reports, the repositories will be requested to propose an action plan describing actions and timelines to address findings.
The following two outstanding issues from the inspections were then presented and discussed with the ACVVR.

1) Variola virus full genomic DNA is transferred to lower containment laboratories at CDC and VECTOR, where it is handled and stored. The issue relates to the authorizations to conduct research and the conditions that should apply, and was felt by the inspection team to fall under the responsibility of the ACVVR. Discussions with the ACVVR confirmed that this was acceptable as long as the full genomic DNA remained under the control of the Collaborating Centres.

2) VECTOR requested formal WHO approval to work with animals in the context of future research campaigns. This was a follow-up from a previous recommendation that "before VECTOR conducts animal experiments the animal facilities should be inspected and approved by WHO-appointed inspectors". Despite assurance that the requested conditions were met, the team did not feel it had the authority to give such approval, and guidance from WHO SMG on this unprecedented issue is currently being sought.

The biosafety inspection visits of 2012 confirmed that this approach allows effective inspections of the repositories, helping assure the wider community that this vital work is being carried out safely and securely, in line with the highest standards of biosafety and biosecurity.

The Smallpox Laboratory Network

Jean-Claude Piffaretti

Interlifescience, 6900 Massagno, Switzerland

In 2010 an ACVVR subgroup produced a report on the concept of a Smallpox Laboratory Network (SLN). In 2011, the ACVVR recommended to implement the SLN. Based on this concept, the main objectives of the SLN programme were defined as follows:

1) to elaborate a scheme for establishing and maintaining a WHO worldwide network of laboratories capable of providing rapid and reliable laboratory screening of clinical samples highly suspected to contain variola virus;
2) to integrate the SLN into a more general diagnostic laboratory network, precisely the WHO Emerging and Dangerous Pathogens Laboratory Network;
3) to identify the molecular diagnostic techniques (PCR and real-time PCR tests) that are currently available for technology transfer to the smallpox laboratories network;
4) to identify a mechanism to ensure the organization of proficiency assays, including the production of positive and negative samples; and
5) to identify a mechanism to ensure the production of a PCR assay for distribution to the SLN.

Unfortunately, due to several reasons including limited resources, in the current year the project did not progress as expected. At present, the candidate regional laboratories are being identified by WHO according to the criteria mentioned in the report. Ultimately, the laboratories will be designated by WHO together with the two WHO Collaborating Centres.

Concerning the diagnostic molecular assays for the detection of variola virus, a very limited number of them is still being available in the market. In addition, they are intended to scientific purposes only, and it is not certain that their production will be continued. An attractive possibility would be to take advantage of two diagnostic assays established by the WHO Collaborating Centres, one in the process of being licensed by the FDA and the other already licensed in the Russian Federation.

Resources are mainly needed to implement proficiency assays, to provide adequate training and for inspections. Without an appropriate funding, it will not be possible to establish the SLN.

Annex 2. Agenda

14th Meeting of the WHO Advisory Committee on

Variola Virus Research

16 to 17 October 2012

Executive Board Room, WHO Headquarters

Geneva, Switzerland

Agenda

16 October 2012

9:00 – 9:15	Opening – Dr K. Fukuda, Assistant Director-General for Health Security and Environment
	Election of Chairman & Rapporteur
	Variola virus reports
9:15 – 9:30	Report of the Secretariat – WHO Secretariat – Dr A. Costa
9:30 – 9:45	Update on research proposals submitted to WHO in 2012 – Dr R. Drillien
9:45 – 9:55	Report on the variola virus collection at the WHO Collaborating Centre Repository in VECTOR, Koltsovo, Novosibrisk, Russian Federation – Prof A. Sergeev
9:55 – 10:05	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 – Dr I. Damon
	Variola virus research 2011 – 2012 Update
10:05 – 10:25	Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus /Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support – (Abstract title: Review of diagnostic development – reagent maintenance and protein based diagnostic development) – Dr I. Damon, Dr V. Olson, CDC
10:25 – 11:00	Tea/Coffee Break
11:00 – 11:20	Discovery of new antiviral for smallpox treatment and prevention/ Development of therapeutic anti-smallpox antibodies/ Assessment of the neutralizing activity of

	vaccine blood sera using live variola virus – Dr L. Bulychev, VECTOR
11:20 – 11:50	Use of live variola virus to determine whether CAST/EiJ mice are a suitable animal model from human smallpox – Dr I. Damon, CDC
11:50 – 12:10	Use of live variola virus to evaluate antivirals against variola – Dr V. Olson, CDC
12:10 – 12:30	Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention – Dr J. Kindrachuk, CDC
12:30 – 14:00	Lunch
14:00 – 14:20	Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of third-generation vaccines – Dr V. Olson, CDC
14:20 – 14:40	Efficacy study of the therapeutic window of oral ST-246® in cynomolgus monkeys infected with variola virus – CDC/Dr A. Goff/SIGA
14:40 – 15:00	New generation smallpox vaccines – Prof S.N. Shchelkunov, VECTOR
15:00 – 15:20	Presentation – Dr L. Borio, FDA
15:20 – 15:50	Tea/Coffee Break
15:50 – 16:10	Progress towards approval of ST-246® – Dr D. Hruby
16:10 – 16:30	Progress on the development of smallpox vaccine IMVAMUNE® – Dr L. Wegner
16:30 – 16:50	Update on LC16m8 vaccine – Dr H. Yokote
16:50 – 17:10	Update on CMX001 – Dr R. Lanier
17:10 – 17:30	Additional presentation
17:30 – 17:50	Additional presentation
17:50 – 18:30	General discussion
18:30 – 19:30	Social event

DAY ONE CLOSE

17 October 2012

9:00 – 9:20	WHO smallpox vaccines: update – Dr I. Pluut
9:20 – 9:50	Update variola virus repositories biosafety inspection visits 2012 –Dr N. Previsani
9:50 – 10.10	The Smallpox Laboratory Network – Dr J-C. Piffaretti
10.10 – 10:30	General discussion and preparation of draft meeting report
10:30 – 11:00	Tea/Coffee Break
11:30 – 12:30	General discussion and preparation of draft meeting report (continued)
12:30 – 13:30	Lunch
13:30 – 15:00	General discussion and preparation of draft meeting report (continued)
15:00 – 15:30	Tea/Coffee Break
15:30 – 16:30	Final discussion and finalization of draft report

ACVVR MEETING CLOSES

Annex 3. List of participants

14th Meeting of the WHO Advisory Committee on Variola Virus Research from 16 to17 October 2012, Executive Board Room, WHO Headquarters, Geneva

MEMBERS OF THE ADVISORY COMMITTEE

Dr Isao Arita*, Chairman, Agency for Cooperation in International Health, 4-11-1 Higashi-Machi, Kumamoto City, JAPAN

Dr Robert Drillien, Directeur de Recherche à l'INSERM, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch Cedex, FRANCE

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

Dr David Evans, Professor and Chair, Medical Microbiology and Immunology, University of Alberta, Alberta, CANADA

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

Dr Ali Shan Khan, Director, Office of Public Health Preparedness and Response, Centers for Disease Control and Prevention, Atlanta, GA, 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, GA, UNITED STATES OF AMERICA

Dr Akhilesh Mishra, Director, National Institute of Virology, Pune, INDIA

Dr Jean-Vivien Mombouli*, Directeur, Département de la Recherche et de la Production, Laboratoire National de Santé Publique, Brazzaville, CONGO

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

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

Dr Anthony John Robinson, Consultant Virologist, CSIRO Sustainable Ecosystems, Michelago NSW, AUSTRALIA

Dr Li Ruan, Director, Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, CHINA

Professor Alexander Sergeev, 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

Professor Geoffrey L. Smith, Wellcome Trust Principal Research Fellow and Head of Department, Department of Pathology, University of Cambridge, Cambridge, UNITED KINGDOM

Professor Robert Swanepoel*, Zoonoses Research Unit, Faculty of Health Sciences, University of Pretoria, SOUTH AFRICA

Professor Muyembe Tamfum*, Director, Institut National de Recherche Biomédicale (INRB) Kinshasa, DEMOCRATIC REPUBLIC OF THE CONGO

Dr Oyewale Tomori*, Regional Virologist for the WHO African Region and Vice-Chancellor, Redeemer's University, Ikeja, Lagos State, NIGERIA

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

Dr Kummuan Ungshusak*, Director, Bureau of Epidemiology, Ministry of Public Health, Nonthaburi, THAILAND

ADVISERS TO THE COMMITTEE

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

Dr Kalyan Banerjee, President, Maharashtra Association for the Cultivation of Science Pune, INDIA

Dr Peter D E Biggins, Defence Science and Technology Laboratory, Porton Down, Salisbury, UNITED KINGDOM

Dr Luciana L. Borio, Assistant Commissioner for Counterterrorism Policy, Office of Counterterrorism and Emerging Threats (OCET), Food and Drug Administration, Silver Spring, MD, UNITED STATES OF AMERICA

Dr Clarissa Damaso, Head, Virus Laboratory Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, BRAZIL

Dr Inger K. Damon, Chief, Poxvirus Section and Rabies Branch, DVRD /NCZUED/ CCID Centers for Disease Control and Prevention, Atlanta, GA, UNITED STATES OF AMERICA

Dr Arthur Jay Goff, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, UNITED STATES OF AMERICA

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Dr Grant McFadden, Department of Molecular Genetics & Microbiology, College of Medicine, University of Florida, Gainesville, FL, UNITED STATES OF AMERICA

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Dr Paul Chaplin*, President, Infectious Disease Division, Bavarian Nordic A/S, Martinsried, GERMANY

Dr Dennis E. Hruby, Chief Scientific Officer, SIGA Technologies Inc., Corvallis, OR, UNITED STATES OF AMERICA.

Dr Randall Lanier, Senior Director of Virology, Office Manager/Executive Assistant, Chimerix Inc., Durham, NC, UNITED STATES OF AMERICA

Dr Lars S. Wegner, Director, Medical Marketing, Bavarian Nordic A/S, DENMARK

Dr Hiroyuki Yokote, Regulatory Affairs, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, JAPAN

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Dr Mauricio Landaverde*, Adviser, Vaccines and Immunization, AMRO/FCH/IM

D**r Hassan El Bushra***, Representative for EMRO

Dr Eugene Gavrilin, CDS Labnet Co-coordinator, Representative for EURO

Representative for SEARO*

Representative for WPRO*

HEADQUARTERS

Mr Alejandro Costa, Team Lead, CED/EVS

Dr Keiji Fukuda, ADG/HSE

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Dr Marie-Paule Kieny*, ADG/IER

Mrs Anne Mazur*, Principal Legal Officer, DGO/DGD/LEG/CCM

Dr Jean-Marie Okwo-Bele*, Director, FCH/IVB

Mrs Marie Sarah Villemin Partow*, Information Officer, ISS/RAS

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Dr Nicoletta Previsani, Team Leader, HSE/IHR/LBS

Dr Cathy Roth, Adviser, HSE/HEA

Dr David Wood*, Coordinator, FCH/IVB/QSS

* Unable to attend