

WHO Advisory Committee on Variola Virus Research

Report of the Thirteenth Meeting

Geneva, Switzerland
31 October–1 November 2011

WHO Advisory Committee on Variola Virus Research

Report of the Thirteenth Meeting

Geneva, Switzerland
31 October–1 November 2011

© World Health Organization 2011

All rights reserved.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either express or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

This publication contains the collective views of an international group of experts and does not necessarily represent the decisions or the policies of the World Health Organization.

Printed by the WHO Document Production Services, Geneva, Switzerland

Table of Contents

Executive summary	3
1. Report from the WHO Secretariat	5
2. Update on research proposals submitted to WHO in 2011	5
3. Update on non-infectious DNA clones of the variola virus held at the National Institute of Communicable Diseases, South Africa	6
4. Comments on the use of variola DNA for a control variola assay evaluation protocol	6
5. Report on the variola virus collection at the WHO Collaborating Centre Repository in VECTOR, Koltsovo, Novosibirsk, Russian Federation	7
6. 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	7
7. Review of protein-based diagnostic development	8
8. Use of live variola virus to evaluate antiviral agents	8
9. Multiplex PCR assay for simultaneous identification of variola virus and other human pathogenic orthopoxvirus species	8
10. Update on smallpox vaccines Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of third-generation vaccines	9
11. Progressive vaccinia and eczema vaccinatum: insights from the laboratory	9
12. Efficacy study of chemically synthesized compounds against orthopoxviruses	10
13. PCR-based diagnostic assay for the multiplex detection of variola virus and agents of viral haemorrhagic fever	10
14. FDA's efforts to facilitate development and approval for smallpox medical countermeasures	11
15. Progress on the development of the smallpox vaccine IMVAMUNE®	11
16. Update on LC16m8 vaccine	12
17. Update on development of hexadecyloxypropylcidofovir (CMX001)	12
18. Progress and challenges on looking at ST-246 in treatment of variola challenged non-human primates	13

19. Progress towards approval of ST-246	13
20. Smallpox non-human primate model refinement	14
21. Additional presentation	14
22. WHO smallpox vaccines: update	14
23. Digitization of the smallpox eradication programme archives	15
24. Variola virus repositories biosafety inspection visits in 2012	15
25. Renewal of the scientific subcommittee for the next three years	16
26. Outline of the key scientific and programmatic agenda for the next three years	16
Annex 1. Summaries of the presentations	18
Annex 2. Agenda	39
Annex 3. List of participants	42

Executive summary

The work done under supervision of the variola virus research programme was summarized and presented to the WHO Advisory Committee on Variola Virus Research. It may be summarized as follows.

The Committee recalled the provisions for research approved by the World Health Assembly in resolution WHA60.1 that "*authorization was granted to permit essential research for global public-health purposes, including further international research into antiviral agents and improved and safer vaccines*".

The Committee received reports from the two authorized repositories of variola virus (VECTOR, in the Russian Federation, and the Centers for Disease Control and Prevention, in the United States of America) on the virus collection that they hold.

All WHO's archives of the Smallpox Eradication Programme have been digitized and uploaded into a dedicated database. Plans are in place to make them available on the Internet.

The Committee noted that two excellent drug candidates (ST-246[®] and CMX001) were in advanced stages of development and that two live attenuated smallpox vaccines showed good safety profiles in human beings and protected against disease induced by several orthopoxviruses in animal models. PCR-based diagnostic tests developed by researchers in the variola virus repositories in the Russian Federation and the United States of America were accurate and sensitive; they could detect variola virus DNA and distinguish it from DNA from other orthopoxviruses.

The Committee agreed to develop the smallpox laboratory network further in collaboration and coordination with the Emerging and Dangerous Pathogens Laboratory Network recently launched by WHO.

Remaining objectives of the research programme were to improve the reproducibility of the non-human primate model for variola virus infection so that additional data on the effectiveness of antiviral agents and vaccines could be generated. Such data would help regulatory agencies to have greater confidence in the effectiveness of these drugs and vaccines against variola virus and therefore help their progress to licensure. The Committee recommended continuation of this work.

Planning for the WHO biosafety inspection visits to the containment facilities in the Russian Federation and the United States of America in mid-2012 is under way. The European Committee for Standardization's Laboratory biorisk management standard, CWA 15793:2008, provides the framework for the forthcoming inspection.

The Committee recommended that the Ad Hoc Committee on Orthopoxviruses should be reconvened to discuss an emergency response to a possible future outbreak of smallpox.

The Committee was informed that the membership of the scientific subcommittee had been renewed.

1. Report from the WHO Secretariat

- 1.1. The WHO Advisory Committee on Variola Virus Research met on 31 October and 1 November 2011 with Professor G.L. Smith as Chairman and Mr D. Bramley as Rapporteur.
- 1.2. Dr K. Fukuda, Assistant Director-General for Health Security and Environment, welcomed participants on behalf of WHO. He recalled the appreciation of the work of the Committee and its regular reports expressed at the Sixty-fourth World Health Assembly in May 2011. The Health Assembly had welcomed progress with the archive project and noted the developments in vaccine stockpiling. Dr Fukuda stressed the importance of developing standard operating procedures for the use of smallpox vaccines in recipient countries. During the influenza A (H1N1) pandemic, because of logistical and legal issues, there were notable challenges in moving stocks of vaccines rapidly to the places they were needed.
- 1.3. Dr Fukuda also recalled the World Health Assembly's appreciation of the development of the network of smallpox diagnostic laboratories in the context of surveillance. In decision WHA64(11), the Health Assembly strongly reaffirmed previous decisions that the variola virus stocks should be destroyed, and agreed that a new date for the destruction should be fixed, and decided to include a substantive item on smallpox eradication: destruction of variola virus stocks on the provisional agenda of the Sixty-seventh World Health Assembly.
- 1.4. The work of the Committee and of the Advisory Group of Independent Experts (AGIES) had been recognized as crucial. Continuing work would be needed on developing the research agenda, stockpiling, and furthering the network of diagnostic laboratories.
- 1.5. Dr P. Formenty presented the report of the Secretariat. The report of the 12th meeting of the Committee had been considered by the Sixty-fourth World Health Assembly. He further summarized progress in developing the smallpox diagnostic laboratory network. An inspection of the two repositories, in the USA and in the Russian Federation, is planned for 2012.

2. Update on research proposals submitted to WHO in 2011

- 2.1. Dr R. Drillien reported on the work of the scientific subcommittee which reviews projects submitted to it for approval. He summarized the project proposals submitted to the subcommittee in 2011. It was noted that of the 10 projects submitted, 8 had been approved. The title of each proposal authorized is given below and a more detailed report of the scientific subcommittee can be found in Annex 1.
 - Project from INSERM, Marseilles, France: Transfer of a variola DNA fragment encoding cytokine response modifier B

- Project from CDC: Antiviral therapy of smallpox and other systemic orthopoxvirus infections
- Project from CDC: Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support
- Project from CDC: Use of live variola virus to develop protein based diagnostic and detection assays specific for variola virus
- Project from CDC: Use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines
- Project from CDC: Use of live variola virus to evaluate antivirals against variola
- Project from CDC: Efficacy study of the therapeutic window of oral ST-246[®] in cynomolgus monkeys infected with variola virus
- Project from Vector: Discovery of new antivirals for smallpox treatment and prevention.

3. Update on non-infectious DNA clones of the variola virus held at the National Institute of Communicable Diseases, South Africa

3.1 Dr R. Swanepoel reported on the non-infectious DNA clones of the variola virus held at the National Institute for Communicable Diseases (NICD) in South Africa. At its 12th meeting in 2010, the Committee had agreed that the NICD could retain cloned variola virus DNA representing up to 20% of the virus genome for diagnostic purposes but that the rest should be transferred to the repository in the Centers for Disease Control and Prevention (CDC) in the USA. The Department of Health of South Africa has now decided to retain up to 20% of a variola virus genome that will be useful in producing diagnostic reagents. The CDC in Atlanta, USA, has received the requested security clearance and transport arrangements for the DNA are being made.

4. Comments on the use of variola DNA for a control variola assay evaluation protocol

4.1 Dr I. Damon reported on research by a private company in the USA which has described inserting 63 nucleotides of variola virus DNA into the genome of an attenuated, but infectious orthopoxvirus. It was noted that this action was inconsistent with the recommendations of the World Health Assembly. Therefore the company would be requested to destroy the reagents and confirm to WHO that this has been done. This incident reaffirmed the need to advertise widely the current recommendations regarding the use of variola virus DNA, and to revisit the recommendations themselves in view of developing technologies and the risks potentially associated with these new technologies.

5. Report on the variola virus collection at the WHO Collaborating Centre Repository in VECTOR, Koltsovo, Novosibirsk, Russian Federation

- 5.1 Dr S. Shchelkunov presented the report of VECTOR which holds a collection of 120 isolates of variola virus obtained from sources in Europe, Asia, Africa, South America, and Eastern Mediterranean (see summary in Annex 1). The collection includes freeze-dried and frozen cultures, and 17 primary specimens isolated from human patients in the past. The total number of registered stored samples is 691.
- 5.2 In 2010, the collection of variola virus was transferred to a new repository located in the building designated for research on variola virus. Aliquot portions of four variola virus strains (6-58, Butler, Ind-3a, Congo-9) were grown in Vero cells and used to assess the antiviral properties of chemically synthesized compounds. After this work was completed, all remaining aliquots were destroyed.
- 5.3 During 2011, no research involving the use of live variola virus was carried out. However, research using the live virus is to be resumed in 2012 in order to assess the antiviral properties of compounds with previously demonstrated antiviral activity against other orthopoxviruses, and to discover primary cell cultures susceptible to variola virus.

6. 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

- 6.1 Dr I. Damon provided an update on work with live variola virus at CDC and the stocks of variola virus retained in the repository. She also explained the time spent in the BSL4 laboratory during recent years (see summary in Annex 1). The virus collection is maintained in two separate freezers, one of which is a back-up facility that has remained largely untouched. Between November 2010 and October 19, 2011 there were nine removals of live variola virus from the repository for WHO-sanctioned protocols. 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.
- 6.2 No new variola virus seed pools were added to the inventory between 2010 and 2011. WHO-approved research activities that have used variola virus from the inventory within the past year have focused on use of non-human primate models for evaluation of antiviral drugs, in vitro analysis of promising antiviral compounds with high orthopoxvirus specificity and efficacy, and evaluation of sera from vaccination regimens to evaluate neutralization of variola virus infectivity.

7. Review of protein-based diagnostic development

- 7.1. Dr K. Karem outlined research activities in variola antigen testing, the development of monoclonal antibodies, viral antigen capture assays, protein microarray development and novel methods for high throughput viral neutralization applicable to variola virus (see summary in Annex 1).
- 7.2. Extension of pilot work on viral capture assays includes testing monoclonal antibody pairs for greater sensitivity for viral detection. Monoclonal antibody 3B6 binds orthopoxvirus protein H3 and is being used in capture assays as well as staining of variola virus for novel high throughput neutralization assay useful for vaccine efficacy studies. Monoclonal antibody E2 reacts specifically to variola virus, but preferentially to gamma irradiated antigen (from which it was generated). Screening of E2 monoclonal antibody against variola virus protein on microarray chips reveals low level binding to several candidate proteins. Binding studies are underway to define the specific target antigen.
- 7.3. Continuing efforts provide options for viral detection and serologic tests to enhance orthopoxvirus testing including variola virus. Ultimately, live variola virus will be employed to define the utility of assays for detection of variola virus.

8. Use of live variola virus to evaluate antiviral agents

- 8.1 Dr V. Olson summarized the orthopoxvirus life cycle and positions within the life-cycle at which various anti-poxvirus drugs act (see summary in Annex 1). Compounds under investigation include tyrosine kinase inhibitors (such as Gleevec®), tecovirimat (ST-246®), bortezomib (Velcade®) and hexadecyloxypropylcidofovir (CMX001). The use of live variola virus to determine these compounds' efficacy in vitro has the potential to identify antiviral agents with unique mechanisms of action at different stages of the viral life-cycle.

9. Multiplex PCR assay for simultaneous identification of variola virus and other human pathogenic orthopoxvirus species

- 9.1 Dr S. Shchelkunov reported on work to develop methods of multiplex polymerase chain reaction (PCR) assay for the detection in a single reaction of any of four species of orthopoxviruses, namely: variola virus, monkeypox virus, cowpox virus and vaccinia virus (see summary in Annex 1). A test kit based on classic multiplex PCR was developed. This product is now licensed in the Russian Federation and is available commercially.
- 9.2 Another project was sought to develop a method of multiplex TaqMan® real-time PCR (MuRT-PCR) for the specific detection and differentiation of DNA of the four different orthopoxviruses in one reaction. This method allowed for greater sensitivity and a shorter time than the first method. A new test kit will be developed based on this method.

Both tests may be used in other diagnostic laboratories in the Russian Federation and can be offered to WHO for monitoring of human orthopoxvirus infections in affected countries.

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

10.1 Dr V. Olson reported on investigations using variola virus to support vaccine development (see summary in Annex 1). Vaccines under evaluation at CDC and elsewhere include modified vaccinia Ankara (MVA) and LC16m8. Variola virus neutralization is important for the evaluation of vaccines that do not elicit a “take”, the traditional measure of vaccine success. The ability of these less reactogenic vaccines to elicit variola virus-specific reactivity will yield fundamental data to help to determine which of the candidate vaccines has the best combination of efficacy and safety. Dr Olson reported on collaborative research into correlations between neutralization assays (variola virus plaque reduction neutralization test [PRNT], vaccinia virus-MVA luciferase neutralization, and vaccinia virus-Dryvax luciferase neutralization) and correlations between neutralization titre and duration/magnitude of virus shedding after human challenge with vaccinia virus-Dryvax.

10.2 Dr Olson further reported on research into the optimization and standardization of a PRNT assay for variola virus extracellular enveloped virus (EEV). This is important because, although the intracellular mature virion represents the majority of progeny virions, EEV is responsible for viral dissemination. Therefore, the ability of a vaccine to induce EEV-neutralizing antibodies is likely to be important for prevention of smallpox.

11. Progressive vaccinia and eczema vaccinatum: insights from the laboratory

11.1 Dr I. Damon reviewed two case studies of investigational use of antiviral agents in severely ill patients suffering from vaccinia virus infection (see summary in Annex 1). The first case was in a 28-month-old child with refractory atopic dermatitis and who had a history of contact with the father who had received the smallpox vaccine. The patient was diagnosed with eczema vaccinatum, and was treated with high doses of vaccinia immune globulin (VIG), off-label cidofovir, and investigational ST-246[®]. Following administration of anti-poxviral therapies the patient recovered.¹

11.2 The second study described a 20-year-old, previously healthy marine who had acute myelogenous leukaemia and who developed progressive vaccinia after receiving smallpox vaccination.² The patient was treated with VIG, the antiviral agents ST-246[®] and CMX001, and imiquimod, an immunomodulatory compound. During the course of the antiviral therapy, virus isolates resistant to ST-246[®] were identified.

¹ Vora S, Damon I, Fulginiti V et al (2008), Severe eczema vaccinatum in a household contact of a smallpox vaccine. *Clinical Infectious Diseases*, 46:1555-1561.

² Progressive vaccinia in a military smallpox vaccinee --- United States, 2009. *Morbidity and Mortality Weekly Report*, 58(19):532-536 (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5819a5.htm>)

Despite becoming critically ill the patient eventually recovered. For further details see Annex 1.

DISCUSSION: Intensive laboratory support was utilized to aid clinical decision making and to adjust dosing of the various therapeutics. The emergence of antiviral resistance was likely related to suboptimal concentrations of the antiviral agent being maintained for a prolonged period. Continued study of these laboratory parameters in preclinical and investigational use of these medical countermeasures will inform their best use. It was suggested, on the basis of comparison with historic cases, that both patients would probably have died without the antiviral treatment.

12. Efficacy study of chemically synthesized compounds against orthopoxviruses

- 12.1 Dr A. Sergeev described testing of new anti-poxvirus compounds, including isoindole derivatives and their close analogues, against orthopoxvirus infections in cell culture and in animal models (see summary in Annex 1). Both variola virus and monkeypox virus were used in the research. Young animals (mice, rabbits, marmots and mini-pigs) were infected with monkeypox virus intranasally or subcutaneously.
- 12.2 The study showed that marmots and mice were susceptible to monkeypox virus whereas the other animals were not. The study in Vero cells showed that one of the 14 compounds under study (referred to as NIOCH-14) had a profound antiviral effect against both variola virus and monkeypox virus that was comparable in potency to that of ST-246. Use of NIOCH-14, at a dose of 60 µg/g intranasally in monkeypox virus-infected mice resulted in 100% protection. It was concluded that marmots and mice can be used as animal models to assess the efficacy of therapeutic and prophylactic compounds against infection caused by monkeypox virus. The compound NIOCH-14 had a profound antiviral effect against both variola virus and monkeypox virus in experiments in vitro and in vivo. The mechanism by which NIOCH-14 inhibits replication of poxviruses is under investigation.

13. PCR-based diagnostic assay for the multiplex detection of variola virus and agents of viral haemorrhagic fever

- 13.1 Dr L. Golightly described a project to develop means of early detection of causative agents of orthopoxvirus infections and viral haemorrhagic fevers (see summary in Annex 1). A multiplex detection assay using several species-specific PCR primers to generate amplicons from multiple pathogens was developed. The resultant fluorescently-labelled ligation products are detected on a universal array enabling simultaneous identification of the pathogens. The assay was evaluated on 53 different isolates associated with viral haemorrhagic fevers as well as variola virus and vaccinia virus. The research showed that the assay was able to detect all viruses tested, including eight representative sequence variants of variola virus from the CDC repository. The assay did not cross-react with other emerging zoonotic agents such as monkeypox virus or cowpox virus, or with six flaviviruses.

DISCUSSION: Comments included the possible future use of the new cowpox virus (calpoxvirus) sequences in this research.

14. FDA's efforts to facilitate development and approval for smallpox medical countermeasures

- 14.1 The US Food and Drug Administration (FDA) is responsible for ensuring the safety, effectiveness, and security of medical products in the USA, including medical countermeasures (MCMs) for smallpox. Dr L. Borio gave an overview of FDA efforts to facilitate the development and review of these countermeasures, including those against smallpox, and to highlight some of the regulatory science challenges associated with the development and regulatory evaluation of smallpox MCMs (see summary in Annex 1).
- 14.2 Regulatory uncertainties related to smallpox MCMs reflect underlying scientific uncertainties. The lack of robust animal models for variola virus is an impediment for the efficient testing of vaccines and antiviral drugs for smallpox. Challenges related to animal model development include the specificity of variola virus for the human host.
- 14.3 In August 2010, FDA launched its Medical Countermeasures Initiative (MCMi), which is intended to strengthen the range of FDA's MCM activities. In September 2011, FDA held a workshop to discuss key scientific issues related to the development and evaluation of next-generation smallpox vaccines. In December 2011, FDA is hosting an advisory committee to discuss pathways for the development of antiviral agents intended to treat smallpox, including the use of appropriate animal models.

DISCUSSION: It was noted that FDA is revising the "Animal Rule" guidance. Other discussions included the possible extension of the shelf-life of vaccines.

15. Progress on the development of the smallpox vaccine IMVAMUNE®

- 15.1 Dr P. Chaplin presented an update on IMVAMUNE® (MVA-BN®) – a live, highly attenuated vaccinia strain that does not replicate in most human cells (see summary in Annex 1). IMVAMUNE® is currently being developed as a stand-alone, third-generation smallpox vaccine.
- 15.2 More than 3400 subjects have been vaccinated with IMVAMUNE® in 16 clinical trials. The subjects include 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 study will begin in 2012 and a licence application for marketing authorization was submitted to Health Canada in 2011.
- 15.3 IMVAMUNE® has a favourable safety profile in both healthy individuals and those with impaired immune function. None of the serious adverse events associated with traditional smallpox vaccines, including myo- or peri-carditis, have been observed following vaccinations with IMVAMUNE®. Clinical studies have shown that the

product induces a strong and long-lasting anti-vaccinia virus immune response in both healthy subjects and at-risk groups.

- 15.4 IMVAMUNE® development is supported by US Government contracts. In May 2010 the first deliveries (of a 20 million dose order) were delivered to the US Strategic National Stockpile, under a pre-Emergency Use Authorization for potential use in HIV-infected subjects following a declared emergency.

16. Update on LC16m8 vaccine

- 16.1 Dr H. Yokote provided participants with an update on the attenuated replication-competent vaccinia virus, LC16m8, which was first licensed in Japan in the 1970s and is currently being stockpiled there (see summary in Annex 1). The LC16m8 vaccine has been given to more than 100 000 infants, and recently more than 8000 members of the self-defence forces, without any severe adverse effects. The clinical efficacy and safety of the vaccine were described and animal studies were summarized. These studies investigated the immunological basis of containment of vaccinia virus in the skin, or dissemination from the skin lesions to distal sites, in order to contribute to the search for safer vaccines against smallpox. The research, using both Dryvax® and LC16m8, showed that both vaccines protect rhesus macaques from a lethal monkeypox virus challenge. Further, it was shown that the risk of adverse events following vaccination of humans with LC16m8 is lower than with the NYCBH vaccine for immunocompromised (CD4 and CD8 depleted) individuals.

DISCUSSION: Dr I. Kurane summarized the main properties of the LC16m8 vaccine. He noted that the vaccine had been selected for stockpiling in Japan.

17. Update on development of hexadecyloxypropylcidofovir (CMX001)

- 17.1 Dr R. Lanier presented an update on CMX001, a lipid antiviral conjugate of cidofovir that is over 200-fold more active in vitro than cidofovir against variola virus (see summary in Annex 1). This compound has proved effective in animal models of orthopoxvirus infections and, under the FDA's animal efficacy rule, is progressing towards a smallpox treatment indication. Progress towards licensure is made in combination with data from human clinical trials of CMX001 against other double-stranded DNA viral infections. Based on animal testing and human dosing in over 600 patients to date, it is expected that a dose of 100 mg CMX001 given orally two times a week may be an effective dose for treatment of variola virus infection based on in vitro and in vivo (animal model and human) data to date. CMX001 is being developed for other clinical indications, and is currently in late-stage clinical development with validation for commercial-scale manufacturing in process and a complete NDA-enabling toxicology package. Placebo-controlled clinical trial data are expected in early 2012 for cytomegalovirus.

DISCUSSION: Issues regarding attenuation of the immune response of concomitantly administered vaccine with cidofovir were discussed.

18. Progress and challenges on looking at ST-246[®] in treatment of variola challenged non-human primates

- 18.1 Dr A.J. Goff described two experiments designed to assess the efficacy of ST-246[®], an antiviral drug designed for the treatment of smallpox (see summary in Annex 1) in cynomolgus macaques. The data were discussed in the context of evaluating the suitability of the variola non-human primate model as it currently exists.
- 18.2 The purpose of the studies was to determine the therapeutic window in monkeys of an orogastric dose of 10 mg/kg/day. The primary measure of efficacy in the studies was intended to be survival. Secondary endpoints included viral DNA levels in the blood, total lesions, clinical observations and clinical chemistry.
- 18.3 In the first study, oral administration of ST-246[®] at the dose indicated reduced disease signs (number of lesions and viral DNA levels). No variola virus-related mortality was noted either in the animals that received ST-246[®] or in placebo-treated animals. Consequently ST-246[®] could not be evaluated for efficacy or prevention of mortality. The second study is ongoing and the existing data are still blinded.

DISCUSSION: Questions were raised regarding the suitability of the model itself. Specifically issues were raised regarding the lack of lethality in the placebo group, the size, weight, and sex of the animals, and the correlation between the number of lesions in an animal and the level of viral DNA in the blood. An issue was also raised concerning the use of purified or non-purified virus.

19. Progress towards approval of ST-246[®]

- 19.1 Dr D. Hruby summarized progress towards the approval of ST-246[®], a compound that was discovered by a traditional high-throughput screening of more than 350 000 compounds for their ability to inhibit the replication of vaccinia virus in vitro (see summary in Annex 1). ST-246[®] is a highly potent, non-toxic and specific inhibitor of orthopoxvirus replication in vitro and in vivo. So far, in more than 40 animal trials using six animal species and six orthopoxvirus pathogens, ST-246[®] has completely protected infected animals against morbidity and mortality. In the best-characterized model, the drug lowers viral load, reduces lesion formation, and protects from death, even when therapy is delayed until after lesions have formed. Similar results have been obtained in the variola virus non-human primate model, despite limitations of the model. A minimum protective dose in the non-human primate has been determined that suggest the proposed human dose provides at least a three-fold safety margin. There have been no treatment failures due to the emergence of resistant variants in infected animals, including non-human primates.
- 19.2 In May 2011, a five-year contract was signed with the United States Department of Health and Human Services to deliver two million courses of ST-246[®] to the strategic national stockpile in the USA.

20. Smallpox non-human primate model refinement

20.1 Dr D. Ulaeto, United Kingdom, summarized progress made by an international panel convened by the CDC to review the variola virus non-human primate model (see summary in Annex 1). The panel examined how animal models of smallpox (focusing on non-human primate studies) can be improved to better resemble human smallpox and/or to evaluate therapeutic interventions. The panel began its work in November 2010 and a draft report is approaching completion. The review covers: the route of challenge; the strain of virus; the strain or origin of the animals (both rhesus and cynomolgus macaques have been used, and a rodent model may now be feasible); the health, age, weight and sex of the animals (a likely recommendation is to use post-pubertal cynomolgus macaques of a single sex, in a weight-range of 3-5 kg); preparation of the virus; and disease outcomes (standardized endpoint scoring systems, clinical scoring sheets, and documentation of lesions). The panel may recommend that standardized protocols for studies using variola virus in non-human primate should also be adopted where possible for future multicentre studies using monkeypox virus in non-human primates.

DISCUSSION: The scope for improving the animal model was acknowledged and support was expressed for further attempts to do so. There was agreement on the importance of matching animals for a restricted range of age, sex, and weight between trials; increasing and standardizing the purity of virus preparations; and standardizing clinical scoring and humane endpoints between trials.

21. Additional presentation

21.1 Dr K. Karem described an event in New York. A caller reported a body found in an iron coffin during a construction project near a church burial ground. A specialist in iron coffins examined the coffin which was of a type popular in the mid-19th century among the wealthier population. As the body showed what looked like smallpox lesions, CDC was contacted. The body was well preserved with plenty of moisture and some remaining soft tissue. Members of the advisory committee were shown photographs of parts of the body with lesions. Samples were taken but all were negative. DNA extraction and PCR were all negative. Further test on dental samples are planned.

DISCUSSION: It was pointed out that Russian tests on the remains of apparent former smallpox victims buried in Siberia also could not retrieve the virus. While the bodies were well preserved by the permafrost, the virus was not.

22. WHO smallpox vaccines: update

22.1 Dr P. Formenty provided an update on the WHO vaccine stocks. Through a virtual stockpile five countries (France, Germany, New Zealand, United Kingdom and USA) have pledged 31 million doses to WHO. The virtual stockpile consists of a mixture of first- and second-generation vaccines. It was noted that the countries guaranteeing the stockpile are predominantly in the northern hemisphere. There is a need to revisit standard operating procedures for all aspects of smallpox vaccine use and distribution.

23. Digitization of the smallpox eradication programme archives

- 23.1 On behalf of Ms M. Villemin, Dr P. Formenty introduced the digitization project for the Smallpox Eradication Programme Archives, which began in September 2009 (see summary in Annex 1). The main scope of the project was the preservation of the paper files and the integration of the scanned archives into a dedicated database with a search engine. As of December 2010, all digitized smallpox archives had been uploaded into the dedicated database. The database includes not only 730 000 paper documents but also maps, photographs and other records.
- 23.2 In early 2011, all WHO staff members (at headquarters and in regional offices) were given access to search and download from the entire database through a SharePoint interface. External researchers and various persons from academia request access to the digitized smallpox files. Currently, visitors to WHO headquarters can be given access to the database while in Geneva. Due to current financial constraints, access to the database has not yet been facilitated through a public web interface.

DISCUSSION: Members of the Committee expressed support for the project which they felt would be a great asset to further research and investigation into smallpox. They urged that the database should be made more publicly accessible as soon as practicable.

It was stressed that the archived materials should be as open as possible to countries, though not necessarily to the general public. It was proposed that, in addition to governments and the members of the Committee, members of AGIES should have access to the archive.

24. Variola virus repositories biosafety inspection visits in 2012

- 24.1 Dr N. Previsani presented a report on a planned inspection visit to the two WHO repositories for variola virus at CDC in Atlanta, Georgia, USA, and at VECTOR in Koltsovo, Novosibirsk, Russian Federation (see summary in Annex 1). The composition of the panel is still being drawn up. The publication in 2008 of the European Committee for Standardization's Laboratory Biorisk Management Standard CWA 15793 provides the framework for the forthcoming inspection. A meeting between the two repositories and biorisk management experts will take place in January 2012 to further refine the inspection process adopted in 2009.

DISCUSSION: Members of the Committee stressed the importance of the inspection process. It was noted that the visits should not be seen as a certification process as this is the business of national regulatory authorities. Questions were asked about the standards of biosafety in the two repositories at the time it was decided that they should continue to hold virus stocks. Other comments related to the timeline for the visits.

25. Renewal of the scientific subcommittee for the next three years

- 25.1 Dr R. Drillien reminded members of the Committee that the task of approving or disapproving projects under the auspices of the Advisory Committee falls to a scientific subcommittee. As three persons were to step down from the subcommittee, three new members were selected to join the subcommittee.

26. Outline of the key scientific and programmatic agenda for the next three years

- 26.1 The Advisory Committee considered development of the smallpox laboratory network (SLN), the emergency response to a smallpox outbreak, and future research with live variola virus that is essential for public health benefit.

It was agreed to develop the smallpox laboratory network (SLN) further. A report from the SLN subcommittee was presented to the Committee at its 12th meeting in November 2010 and discussions were ongoing within the WHO regional offices to identify appropriate laboratories for this network. It was considered desirable that the same diagnostic tests for variola virus should be used by these laboratories. It was also agreed that the SLN subcommittee would consider which diagnostic kit(s) should be selected and would develop standardized procedures for sampling and would report back to the Committee meeting in 2012. In addition there should be discussions and coordination between the SLN subcommittee and the Emerging and Dangerous Pathogens Laboratory Network (EDPLN) a network recently launched by WHO.

- 26.2 The Committee then addressed the emergency response to a possible future smallpox outbreak. It was recognized that the International Health Regulations (2005) provide a framework for global action and it was agreed that the Ad Hoc Committee on Orthopoxviruses should be re-convened to discuss an emergency response and to report back to the Committee at its 14th meeting in 2012. It was noted that the operational response would include utilization of experience in diagnosis of clinical smallpox.
- 26.3 Finally, Committee members discussed future essential research with live variola virus that is necessary for public health benefit. The Chair reminded the Committee that research was focussed on: (i) two antiviral agents that targeted variola virus at different stages in its replication cycle, (ii) a safer and effective vaccine for smallpox, and (iii) a diagnostic kit that could detect variola virus accurately and rapidly. Excellent progress had been made towards all these objectives.

First, two excellent drug candidates (ST-246[®] and CMX001) are under advanced development. ST-246[®] was effective against all orthopoxviruses tested (including variola virus and monkeypox virus) and, although it had not yet been licensed, it had been purchased recently by the US Government. CMX001 also shows great promise against orthopoxviruses, and is also under development for treatment of infections by other double-stranded DNA viruses. It was noted that in addition to preventing disease caused by several orthopoxviruses in animal models, both drugs had been used to treat life-threatening vaccinia virus infections in two human patients and had, in

combination with vaccinia immune globulin, resulted in their survival, which in the absence of effective antiviral agents seemed improbable.

Secondly, two live attenuated smallpox vaccines, based on vaccinia virus strains LC16m8 and MVA, showed good safety profiles in humans and protected against disease induced by several orthopoxviruses in animal models. In addition, LC16m8 was already stockpiled and licensed for use in Japan, and MVA had been purchased and stockpiled in the USA.

Thirdly, PCR-based diagnostic tests had been developed in both the CDC in the USA and VECTOR in the Russian Federation. Both tests were accurate and sensitive and could detect variola virus DNA and distinguish this from DNA from other orthopoxviruses. The kit from VECTOR had been licensed for use in the Russian Federation, and was available for sale commercially. A standardized assay from CDC, used in the US “Laboratory Response Network”, had been shown to be robust when used in emergency conditions to diagnose cases of monkeypox virus or vaccinia virus infection and distinguish these from infection by variola virus. In addition, minor updates to the PCR-based diagnostics were considered desirable so that these tests would distinguish against additional strains of cowpox virus for which sequence data had become available recently.

A remaining objective for the next three years was to improve the reproducibility of the non-human primate model for variola virus infection so that additional data on the effectiveness of antiviral agents and vaccines against variola virus could be generated. Such data would help regulatory agencies to have greater confidence in the effectiveness of these drugs and vaccines against smallpox and therefore help progress to licensure. To enhance the animal model it was recommended that the age, weight, sex and disease status of the non-human primates should be standardized, and that only purified virus, rather than crude lysates of infected cells, should be used.

Annex 1. Summary of presentations

Update on research proposals submitted to WHO in 2011

Scientific subcommittee members: **Robert Drillien, Mariano Esteban, Grant McFadden, Hermann Meyer, Akhilesh Chandra Mishra, Jean-Claude Piffaretti, Tony Robinson, Oyewale Tomori.**

27 January 2011: proposal submitted by INSERM, Marseille, France - approved

- Transfer of a variola DNA fragment encoding cytokine response modifier B from CDC to INSERM

28 February 2011: proposal submitted by CDC, Atlanta - approved

- Antiviral therapy of smallpox and other systemic orthopoxvirus infections

16 March 2011: 5 proposals submitted by CDC, Atlanta - 4 approved

- Use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support
- Use of live variola virus to develop protein-based diagnostic and detection assays specific for variola virus
- Use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines
- Use of live variola virus to evaluate antivirals against variola

29 April 2011: 2 proposals submitted by VECTOR, Kolstovo - 1 approved

- Discovery of new antivirals for smallpox treatment and prevention

10 October 2011: proposal submitted by CDC, Atlanta - approved

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

Update on non-infectious DNA clones of the variola virus held at National Institute of Communicable Diseases, South Africa

Robert Swanepoel

National Institute for Communicable Diseases, South Africa

At the 2010 ACVVR meeting agreement was reached that the National Institute for Communicable Diseases (NICD) in Johannesburg would be allowed to retain some of the variola clones in their possession for diagnostic purposes, but that these clones should not represent more than 20% of the variola genome.

The remaining clones were to be offered to the CDC Atlanta Repository, if they did not already have duplicate material in their possession. Dr Damon duly confirmed that they did not have duplicate material at CDC Atlanta, but indicated that they would have to obtain security clearance to acquire and transport the material. As a result of an extended delay in obtaining the required clearance, the Department of Health in South Africa agreed instead to the destruction of the clones. A date for destruction was scheduled by Professor Paweska, NICD, for late in 2011, to be witnessed by a WHO representative. However, just prior to the 2011 ACVVR meeting, Dr Damon confirmed that CDC had now obtained the clearance to acquire and transport the clones with an additional one month needed to finalise transport arrangements.

Report on the variola virus collection at the WHO Collaborating Centre Repository in VECTOR, Koltsovo, Novosibirsk, Russian Federation

Alexander N. Sergeev

FBRI SRC VB VECTOR, Koltsovo, Novosibirsk region, Russia

Organization of, and experimentation with, the variola virus (VARV) collection at the WHO Collaborating Centre (WHOCC) at SRC VB VECTOR, Russian Federation are 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 these standards. Plans have been developed for anti-epidemic measures and for response to accidents. Emergency teams have been established for activation in the event 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 691.

In 2010, the entire collection of variola virus was transferred to a new repository, a BSL-4 facility located in the building, which had been designated for experimentation and research on variola virus.

In 2010, aliquots of four variola virus strains (6-58, Butler, Ind-3a, Congo-9) were grown in Vero cells and used to assess the antiviral properties of chemically synthesized compounds. Following the completion of this work, all remaining aliquots were destroyed.

No research involving the use of live variola virus has been performed in 2011.

Research using live variola virus will resume in 2012 to:

- assess the antiviral properties of compounds with previously demonstrated antiviral activity against other orthopoxviruses;
- assess the antiviral properties of fully human antibodies with previously demonstrated antiviral activity against other orthopoxviruses;
- study the efficacy of tablet formulations of anti-smallpox vaccines, being developed in Russia, in live variola virus neutralization reaction;
- discover primary cell cultures susceptible to variola virus.

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

Victoria Olson, Kevin Karem, Paul Hudson, Christine Hughes, Inger Damon

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

The WHO Collaborating Centre for Poxviruses in Atlanta, GA continues to maintain one of two consolidated, international collections of variola 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 2010 and 2011. WHO-approved research activities which have utilized variola virus from the inventory within the last year have focused on use of non-human primate models for evaluation of antiviral therapy, in vitro analysis of promising antiviral compounds with high orthopoxvirus specificity and efficacy, and evaluation of sera from vaccination regimens to evaluate efficacy based on variola neutralization.

In the period from November 2010 to 19 October 2011 there were nine removals of variola from the repository for WHO-sanctioned protocols.

Review of protein-based diagnostic development

Inger Damon, Kevin Karem, Victoria Olson

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

This protocol was last renewed (March 2011) and is valid through to December 2011.

Purpose/ Public health relevance

The ability to validate nucleic acid-based and protein based diagnostic capacity is critical for early detection and recognition of smallpox if reintroduction becomes a reality. The consequences of either false negatives, or false positives, could result in a significant breakdown in public health infrastructure as well as general societal unrest. The need to improve and maintain the WHO Collaborating Centre (WHO CC) variola DNA stocks, and variola antigen stocks is important for future diagnostic research and for the role of CDC as a WHO CC for Smallpox and other Poxvirus Infections.

Results

DNA diagnostics

In 2010, several strains of cowpox virus showed cross reactivity to previously validated variola-specific signature (VRL1). Identification and characterization of unique variola virus signature sequences are being assessed for specificity and sensitivity of variola virus specific assays. The United States Laboratory Response Network (LRN) is also working with CDC in evaluating these assays.

Protein diagnostics

Since late 2010, studies have been continuing on monoclonal antibody characterization, viral antigen capture assays, protein microarray development and novel methods for high throughput viral neutralization applicable to variola virus. Additional monoclonal antibodies have been tested for antigen capture using vaccinia and also piloted using variola antigen. Limits of detection (LoD) are currently around 104 pfu/ml with the use of fluorescent techniques appearing to enhance LoD. Protein microarrays show specific reactivity of monoclonal antibodies, with the promise of identifying additional reagents for specific assays. Pilot tests are underway of a novel high throughput viral neutralization assay that may be applied to variola virus for vaccine efficacy studies.

Discussion/future directions

Despite the fact that the variola-specific target was designed within a highly conserved gene, the region was found to not be unique to variola virus, with a similar sequence also found within the human pathogen cowpox virus. Recent efforts will allow more specific assays for validation and ultimately dissemination for variola testing. Protein based assays are still under refinement and additional studies of characterization of monoclonal antibodies and fluorescent assays will enhance diagnostic options. These continuing efforts will provide options regarding protein-based viral detection as well as serologic tests to enhance orthopoxvirus testing.

Methods

All work with live variola virus is performed at maximum containment (biosafety level 4) under the Terms of Reference of the WHO CC for Smallpox and Other Poxvirus Infections at the WHO CC in Atlanta, GA USA.

Use of live variola virus to evaluate antiviral agents

Inger Damon, Kevin Karem, Victoria Olson

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

Additional external collaborators: Jeffrey Langland, Ph. D., Mark Prichard, Ph.D., Michele Barry, Ph.D.

This protocol was last renewed (March 2011) and is valid through to December 2011.

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. 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 animal model characterization of their activity against live variola virus infection. Several studies have demonstrated that cellular targeted chemotherapy may be protective to treat variola virus infection or enable prophylaxis. Tyrosine kinase inhibitory compounds, such as CI-1033 and Gleevec[®], evaluated for or in use for treatment of human cancers, have shown promise in animal model treatment studies of systemic orthopoxvirus infection; the compounds did not prevent viral replication but dramatically diminished variola virus extracellular enveloped virus (EEV) release, as shown by a viral comet plaque reduction assay. Development of antivirals or immunotherapeutics that directly target variola virus components of infection should continue such as studies of cidofovir and its derivatives, which likely target the DNA polymerase, ST-246, which targets a viral particle assembly protein, and other compounds yet to be described in the literature which would need to be screened for their safety and efficacy against variola virus infection in culture or animal models.

Research within Jeffrey Langland's laboratory has "rediscovered" a carnivorous plant, *Sarracenia purpurea*, as having anti-orthopoxvirus activity. Multiple historic reports exist on *S. purpurea*'s successful treatment of smallpox outbreaks in the North American continent. Langland's group has demonstrated that *S. purpurea* extracts effectively inhibit viral replication and the viral-induced cytopathic effects of various orthopoxviruses. At doses where virus replication was inhibited (25 µl/ml media), little to no cellular toxicity was observed. As presented to the WHO Advisory Committee on Variola Virus Research in November 2010, a dramatic decrease in early gene expression from variola virus was seen with as little as 10 µl *S. purpurea* extract/ml media. Future work will focus on further characterization of the *S. purpurea* extract: identification of the active component within the extract (which may necessitate further anti-variola evaluation), and efficacy within an animal model of systemic orthopoxvirus disease.

There are other interesting potential anti-variola compounds to evaluate; at least two new promising classes (5-substituted 4'-thiideoxyribonucleosides, proteasome inhibitors) of antivirals have shown promise against other orthopoxviruses, such as vaccinia and cowpox viruses. This work has been approved through to December 2011, but has not yet been initiated. The use of live variola virus to determine these compounds *in vitro* efficacy has the potential to identify antiviral agents with unique mechanisms of action at different stages of the viral life cycle. In 2009, the Barry group published a manuscript that illustrated the potential use of a

currently licensed compound, Velcade[®], as a vaccinia, ectromelia and cowpox virus antiviral by virtue of its inhibitory effect on the proteasome. [Teale A, et al. Orthopoxviruses require a functional ubiquitin-proteasome system for productive replication. *J Virol.* 2009 Mar;83(5):2099-108.] The compound was able to prevent formation of viral replication factories, indicating action at a unique stage of the viral life cycle. Efforts will 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[®]. It is likely that an extension will be needed to acquire the compound and conduct efficacy testing.

Multiplex PCR assay for simultaneous identification of variola virus and other human pathogenic orthopoxvirus species

Sergei Shchelkunov, Gavrilova E.V., Shcherbakov D.N., Maksyutov R.A., Sergeev A.N

Department of Genomic Research, State Research Centre of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, the Russian Federation

The genus *Orthopoxvirus* of the Poxviridae family includes four human pathogenic species: variola virus (VARV), monkeypox virus (MPXV), cowpox virus (CPXV), and vaccinia virus (VACV). Work has been ongoing to develop methods of multiplex PCR assay for the detection of any of these four orthopoxviruses species, in a single reaction.

As a first step, a test kit was developed based on classic multiplex PCR. Five pairs of primers (for VARV, MPXV, CPXV, VACV, and a pair of genus-specific primers) were used in one polymerase chain reaction, in the course of which products of different sizes emerged that were typical of each specific orthopoxvirus species. The genus-specific pair of oligonucleotides was used as an internal PCR control to detect orthopoxvirus DNA in a sample in electrophoresis and to discriminate it from other orthopoxvirus genera. The specificity of the MPCR method developed was assessed using a DNA panel of 59 orthopoxvirus strains. This method underlies a test kit (reagents kit), “VECTOR-MPCR-SMALLPOX”, covered by Registration Certificate # FSR 2010/09002 and licensed for manufacture, sale, and use in the Russian Federation.

A method has recently been developed, based on real-time PCR, of multiplex TaqMan real-time PCR (MuRT-PCR) for the specific detection and differentiation of DNA of 4 human pathogenic orthopoxviruses in one reaction. In conducting MuRT-PCR-analysis of orthopoxviral DNA, four species-specific pairs of oligonucleotide primers were used simultaneously with four species-specific hybridization tests with various fluorescent dyes and the respective quencher agents. The specificity and sensitivity of the method developed was assessed by analyzing the DNA of 29 strains of 6 orthopoxvirus species, as well as of DNA samples isolated from historic clinical material from human smallpox cases, of experimental material recovered from mice, infected with CPXV, and from marmots infected with MPXV. This procedure allows for greater sensitivity and a shorter time than the former MPCR method we developed to conduct species-specific gene diagnosis of human pathogenic orthopoxviruses.

The tests developed can be made available to WHO for the purposes of monitoring human orthopoxvirus infections in relevant countries.

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

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

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

This protocol was last renewed (March 2011) and is valid through to December 2011.

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 vaccinia vaccines (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 endpoint for the evaluation of vaccines.

However, a vaccine's ability to neutralize the extracellular enveloped virus (EEV) form of virus may be critical for vaccine efficacy since EEV is important for viral dissemination and ultimate disease pathogenesis [Smith et al. *J. Gen. Virol.* 2002, 83: 2915-31]. 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) were presented to the WHO Advisory Committee on Variola Virus Research (ACVVR) in November 2010. A manuscript detailing our results and analysis has been composed. The second collaboration is with researchers at Harvard University (DMID 05-0010). The study involved 72 subjects randomized into six groups. In 2010, the results on the ability of the higher dose subcutaneous (SC) and intradermal (ID) arms (Groups D and E) to neutralize variola virus MV, were presented. Sera from five to seven different time points were evaluated to understand the kinetics of the immune response over time following MVA vaccination. Individual variability was noted in the overall kinetics of the anti-variola immune response, but an anamnestic boost was seen in all those “challenged” with standard Dryvax[®] vaccination. The results, presented to the WHO ACVVR in November 2010, identified 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.

Optimization and standardization of variola virus EEV PRNT assay

Although IMV represents the majority of progeny virions, EEV is believed to be responsible for viral dissemination and pathogenesis [Smith et al. *J. Gen. Virol.* 2002, 83: 2915-31]. Therefore, the ability of a vaccine to engender resistance to EEV will be critical for treatment/prevention of smallpox. Plaque reduction neutralization experiments using vaccinia virus were used as the basis of our variola virus EEV PRNT assay [M. Benhnia, et al. *J. Virol.* 2009, 83: 1201-1215]. Effective inactivation ($\geq 90\%$) of vaccinia virus EEV particles was enhanced by the presence of host complement plus polyclonal antibodies.

Progressive vaccinia and eczema vaccinatum: insights from the laboratory

Edith R. Lederman, Whitney Davidson, Harold L. Groff, Scott K. Smith, Tyler Warkentien, Yu Li, Kimberly A. Wilkins, Kevin L. Karem, Rama S. Akondy, Rafi Ahmed, Dennis E. Hruby, Wendy P. Painter, Kimberly L. Bergman, Jeffrey Cohen, and Inger K. Damon

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

In May 2009, the Morbidity and Mortality Report “Progressive Vaccinia in a Military Smallpox Vaccinee - United States, 2009” was published online.¹

The report described the clinical presentation and treatment of a severe case of progressive vaccinia in a patient diagnosed and treated for acute myelogenous leukemia shortly after receiving smallpox vaccine.

This presentation will describe some of the laboratory studies utilized to support treatment of the patient with a licensed biologic (VIG-IV), off-label use of an immunomodulatory compound (imiquimod), and investigational antivirals (ST-246[®] and CMX001). These include measurements of blood and tissue quantitative viral DNA, skin lesion viral loads, white blood cell counts, pharmacokinetic measurements, humeral anti-orthopoxvirus responses, cellular anti-orthopoxvirus responses, and assessments of antiviral resistance. The patient was critically ill, and both neutropenic and lymphopenic for an extended period of time. A decrease of viable virus, followed by viral DNA, was associated with laboratory observations of normalization of lymphocyte counts, and clinical improvement. The period of treatment with therapeutics targeted for progressive vaccinia ran from 5 March to 12 May.

¹ Progressive vaccinia in a military smallpox vaccinee --- United States, 2009. *Morbidity and Mortality Weekly Report*, 58(19):532-536 (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5819a5.htm>)

Efficacy study of chemically synthesized compounds against orthopoxviruses

Artemiy Sergeev, Ar.A. Sergeev, L.E. Bulychev, O.V. Pyankov, Al.A. Sergeev, A.S. Kabanov, S.A. Bodnev, Yu.V. Tumanov, I.A. Yurganova, A.P. Agafonov, A.N. Sergeev

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

In spite of the success of the global Smallpox Eradication Programme, smallpox still remains an important concern to public health. Moreover, human-pathogenic orthopoxvirus infections, such as monkeypox, cowpox, and buffalopox, continue to pose a threat to human health, causing outbreaks in different parts of the world. Following the end of the eradication programme, mass vaccination against smallpox ceased. Today more than a half of world's population has no specific immunity against orthopoxvirus infections. It is therefore essential that the public health sector develops high-performance and safe therapeutic and preventive compounds against diseases caused by human-pathogenic orthopoxviruses. The focus of this particular research programme was on studying the therapeutic and preventive effectiveness of compounds against human pathogenic orthopoxviruses using cell culture and different animal models.

Variola virus (VARV) and monkeypox virus (MPXV) strains were used in this research. Young animals (mice, rabbits, marmots and miniature pigs) were intranasally infected with MPXV. Chemically synthesized compounds (14 pcs.), isoindole derivatives and their close analogs, were used as therapeutic and preventive compounds against MPXV.

The study showed that marmots (ID_{50} and $LD_{50} = 2.2$ lg PFU in intranasal infection) and mice ($ID_{50} = 4.8$ lg and $LD_{50} > 5.0$ lg PFU in intranasal infection) were susceptible to MPXV, taking into account the external clinical signs of disease and lethality. The marmots developed strongly pronounced external clinical signs such as pock-like rashes on the skin and mucous membranes, purulent discharge from the nasal cavity, hypotaxia, fever, disheveled hair etc. The illness in mice was associated with purulent conjunctivitis, blepharitis, disheveled hair etc. The other animals (rabbits and miniature pigs) had neither external clinical signs of disease nor lethal effect, with the MPXV infectious doses used. At the same time, the susceptibility rate of mice to MPXV, based on probable virus accumulation in lungs (ID_{50}), made up 2.35 lg PFU per animal, whereas the maximal MPXV accumulation rate in the lungs of mice was observed on day 7 post infection.

The efficacy study of therapeutic and preventive compounds in Vero cells in assessing the index selectivity showed that one of the 14 compounds under study (NIOCH-14) had a profound antiviral effect in relation to both VARV and MPXV, which was comparable in terms of potency to that of the well-known compound, ST-246[®]. When using this compound, following a therapeutic and preventive scheme, at a dose of 60 ug / g in mice infected intranasally with 10 LD_{50} MPXV, protection was achieved in 100% of the animals.

It can be concluded that marmots and mice can be used as model animals to assess the efficacy of therapeutic and preventive compounds against infection caused by MPV. The compound NIOCH-14 had a profound antiviral effect against both VARV and MPXV in experiments in vitro and in vivo.

PCR-based diagnostic assay for the multiplex detection of variola virus and agents of viral haemorrhagic fever

Linnie Golightly

Cornell University, United States of America

Infectious agents that fall within the Centers of Disease Control and Prevention (CDC) designated category A, pose a major risk to national security and require special action for public health preparedness. They include viruses that cause viral haemorrhagic fever (VHF) syndrome as well as variola, the agent of smallpox. VHF is characterized by haemorrhage and fever with multi-organ failure leading to high mortality and morbidity. Smallpox, once a major scourge to the human population, has been eradicated for decades making it a particularly serious threat if released deliberately into an essentially non-immune world population. Early detection of the causative agents and ability to distinguish them from other pathogens is essential in containing outbreaks, implementing proper control measures and preventing morbidity and mortality.

A multiplex detection assay has been developed that uses several species-specific PCR primers to generate amplicons from multiple pathogens; these are then targeted in a ligase detection reaction (LDR).

The resultant fluorescently-labeled ligation products are detected on a universal array enabling simultaneous identification of the pathogens. The assay was evaluated on 53 different isolates associated with VHF (Ebola virus, Marburg virus, Crimean Congo haemorrhagic fever virus, Lassa fever virus, Rift Valley fever virus, dengue virus and yellow fever virus) as well as variola and vaccinia (the agent of smallpox and its vaccine strain, respectively). The assay was able to detect all viruses tested including 8 representative sequence variants of variola virus from the CDC repository.

It does not cross react with other emerging zoonoses such as monkeypox or cowpox, or six flaviviruses tested (St. Louis encephalitis virus, Murray Valley encephalitis virus, Powassan virus, tick-borne encephalitis virus, West Nile virus and Japanese encephalitis virus).

FDA's efforts to facilitate development and approval for smallpox medical countermeasures

Luciana Borio

US Food and Drug Administration, United States of America

The US Food and Drug Administration (FDA) is responsible for ensuring the safety, effectiveness, and security of medical products, including medical countermeasures (MCMs). FDA also works to help foster the development of smallpox MCMs – with a goal towards FDA approval¹ – as well facilitating timely access to them in the event of an emergency primarily through its engagement with the US Department of Health and Human Services' (HHS) Public Health Emergency Medical Countermeasures Enterprise (Enterprise). This presentation provides an overview of FDA efforts to facilitate the development and review of MCMs, including smallpox MCMs, and to highlight some of the regulatory science challenges associated with the development and regulatory evaluation of smallpox MCMs. All of FDA's regulatory mechanisms for ensuring access to safe and effective MCMs require risk-based decisions that are founded on the evaluation of 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 the development of informative animal models has been arduous.

In August 2010, FDA launched its Medical Countermeasures Initiative (MCMi), which is intended to strengthen FDA's MCM activities. The MCMi addresses key challenges in three areas: (1) enhancing the regulatory review process for the highest priority MCMs and related technologies; (2) advancing regulatory science for MCM development; and (3) modernizing the legal, regulatory, and policy framework to facilitate MCM development, access, and ensure an effective public health response. FDA continues to work closely with product sponsors – through mechanisms such as interactive review – to facilitate the successful development and approval of smallpox MCMs. In addition, FDA conducts workshops and Advisory Committee meetings to discuss regulatory challenges and gather stakeholder input into pathways for the successful development and approval of smallpox MCMs.

Challenges related to animal model development include the issues related to the specificity and pathogenicity of variola virus (VARV) for the human host (e.g. differential susceptibility and disease course and severity in animal models, ability to bridge data generated in animal models to man), study design considerations (e.g. viral strain, timing and duration of intervention, appropriate endpoints), and other logistical and regulatory considerations (e.g. BSL-4 requirements for specific studies). Regulatory uncertainties related to smallpox MCMs reflect scientific uncertainties. Diagnostics, drugs, and vaccines each present their own unique uncertainties and challenges. FDA is working with MCM developers and US government partners to resolve scientific uncertainties where feasible.

The identification and resolution of the scientific gaps that hinder smallpox MCM development and approval is essential and will require collaborative efforts. FDA has a renewed commitment to working with partners to solve these challenges.

¹ The term "approval" refers to "FDA-approval, licensure, or clearance" under sections 505, 510(k), or 515 of the Federal Food, Drug, and Cosmetic Act or section 351 of the Public Health Service Act.

Progress on the development of the smallpox vaccine IMVAMUNE®

Paul Chaplin

Bavarian Nordic A/S, Denmark

Currently developed as a stand-alone, third generation smallpox vaccine, IMVAMUNE® (MVA-BN®) is a live, highly attenuated vaccinia strain, which does not replicate in human cells.

More than 3400 subjects have been vaccinated with IMVAMUNE®, in 16 completed or on-going clinical trials, including 1000 subjects from risk groups with contraindications for conventional smallpox vaccines, i.e. people infected with HIV or diagnosed with atopic dermatitis. A Phase III study will commence in 2012, while a license application was submitted to Health Canada in 2011, potentially resulting in the first marketing authorization for IMVAMUNE® in 2012.

IMVAMUNE® has shown to possess an equally favourable safety profile in healthy individuals, as well as in populations with impaired immune function. Importantly, none of the serious adverse events, including myo / pericarditis, associated with traditional smallpox vaccines has ever been observed following vaccinations(s) with IMVAMUNE®.

Clinical studies have shown IMVAMUNE® to induce a strong, long-lived vaccinia-specific immune response in at-risk groups that is comparable with healthy subjects.

While the peak immune responses induced by IMVAMUNE® are non-inferior to traditional vaccines like Dryvax®; IMVAMUNE® induces a faster response compared to traditional smallpox vaccines, which translates to a faster onset of protection in relevant animal challenge models.

The current data fully support IMVAMUNE® as a suitable candidate for use in the general adult population, including those with contraindications to conventional smallpox vaccines.

IMVAMUNE® development is supported by US Government contracts DMID N01-AI-30016, DMID N01-AI-40072, HHSO100200700034C and in May 2010 the first IMVAMUNE® delivery (of a 20 million dose order) was made to the US Strategic National Stockpile, under a pre-Emergency Use Authorization (EUA) for the potential use in HIV infected subjects following a declared emergency.

Update on LC16m8 vaccine

Hiroyuki Yokote

The Chemo-sero-therapeutic Research Institute, Kumamoto, Japan

In the 1970's, an attenuated replication-competent vaccinia virus, LC16m8, was developed from the Lister strain by serial passaging in primary rabbit kidney cells. LC16m8 has demonstrated a low neuro-virulence and a good protective efficacy in animal models. The LC16m8 vaccine has been given to >100 000 infants and recently, to over 8000 members of the armed forces without any severe adverse effects.

Investigations have been ongoing into the immunologic basis of containment of vaccinia in the skin or dissemination from the skin lesions to distal sites in order to examine safer vaccines against smallpox.

Specifically, the T- or B-cells of macaques were systematically depleted and the animals were then vaccinated with either Dryvax[®] or the LC16m8 vaccine. The results indicated that B-cell depletion did not affect the size of skin lesions induced by either vaccine. However, while depletion of both CD4+ and CD8+ T-cells made no adverse effects on LC16m8-vaccinated animals, it caused disseminated vaccinia in macaques immunized with Dryvax[®]. As both Dryvax[®] and LC16m8 vaccines protect macaques from a lethal monkeypox challenge, the results indicated that LC16m8 vaccine is a safer and effective alternative vaccine to NYCBH vaccine for immune compromised individuals.

Update on development of hexadecyloxypropylcidofovir (CMX001)

Randall Lanier

Chimerix Inc., Durham NC, United States of America

Antiviral therapeutics are needed for use in the event of a smallpox outbreak. CMX001 is a lipid antiviral conjugate with activity against the causative agent of smallpox, variola virus (VARV). It is in late stage development with over 600 patients evaluated for multiple viral disease indications, commercial scale manufacturing validation in process, and a complete NDA-enabling toxicology package. CMX001 readily enters cells, where the lipid moiety is cleaved to release cidofovir (CDV). CDV is then phosphorylated to its diphosphate form (CDV-PP) which mimics a nucleoside triphosphate and acts as a selective inhibitor of DNA polymerases. Through this mechanism, CMX001 inhibits orthopoxviruses, adenoviruses and herpesviruses. In contrast to CDV, CMX001 is administered orally, and due to its differing pharmacokinetic profile, there is no indication of CDV-like, dose-limiting renal toxicity based on data obtained from patients in placebo controlled and expanded access emergency treatment human clinical trials.

For orthopoxviruses, CMX001 is considerably more active than CDV with the ratio of the EC₅₀ for CDV/EC₅₀ for CMX001 ranging from 24-fold for ectromelia virus to 271-fold for VARV. The increased activity of CMX001 relative to CDV is attributed to the more efficient cellular uptake of CMX001 facilitated by the lipid chain in combination with conversion to the active antiviral CDV-PP. The net effect is that more active antiviral is delivered intracellularly with less systemic exposure to the parent drug. The broad spectrum activity of CMX001 against various species of orthopoxviruses was anticipated based on the mechanism of action of the drug (inhibition of the virally encoded polymerase by CDV-PP) and the high level of homology for this enzyme seen within the orthopoxvirus genus. The amino acid sequences of the catalytic subunit of the polymerase for orthopoxviruses have sequence identity ranging from 98.2% to 99.1%. Given the conservation of this region it is not surprising that resistance to CDV is slow to develop, requires multiple mutations for high level resistance, and is associated with decreased viral fitness.

Chimerix is developing CMX001 for the treatment of smallpox under the “Animal Rule.” In addition, Chimerix is developing CMX001 for other clinical indications (e.g. prevention of cytomegalovirus (CMV) disease and pre-emption of adenovirus (AdV) disease in hematopoietic cell transplant recipients) using more traditional regulatory pathways to approval that are based on the demonstration of human safety and efficacy in the intended indication, rather than in surrogate models. Given the regulatory and scientific complexities of the “Animal Rule”, the considerable human clinical data that have been and will continue to be generated for CMX001 by Chimerix for these other indications (e.g., CMV and AdV) will provide important supportive clinical safety and efficacy data for CMX001 for submission in a New Drug Application.

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

Progress and challenges on looking at ST-246® in treatment of variola challenged non-human primates

Jay Goff

U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), United States of America

Smallpox is an acute, contagious, disease in humans caused by the variola virus (VARV, an orthopoxvirus) and is marked by fever and a distinctive progressive, disfiguring skin rash. A worldwide vaccination effort eradicated the disease but did not eliminate the etiological agent, variola virus. While vaccination is effective at preventing infection, only live virus vaccines exist and they are contraindicated for a large segment of the population. New therapeutics are necessary to treat and prevent pathogenic orthopoxvirus infections. Animal models of orthopoxvirus disease are the only method available to evaluate drugs for the treatment of smallpox. Infection of non-human primates via intravenous injection of 1×10^8 pfu of variola virus (Harper strain) induces a disease similar to smallpox in humans from the point of secondary viremia, but does not replicate smallpox disease development in humans.

ST-246® is an anti-viral drug designed for the treatment of smallpox. The safety and efficacy of ST-246® were evaluated in cynomolgus monkeys infected with variola virus in two separate experiments.

The purpose of these studies was to determine the therapeutic window in the cynomolgus monkey of an orogastric dose of 10 mg/kg/day ST-246®. The dose of 10 mg/kg/day is approximately equivalent to the proposed human oral dose (400 mg/day) based on body surface area conversion. Monkeys were intravenously challenged with variola virus on study day 0. For therapeutic treatment of variola in the monkey, animals were administered ST-246® at the first sign of skin lesions or on pre-determined days post-infection. The primary measure of efficacy in these studies was survival. Secondary endpoints included viral DNA levels in the blood (measured as time-weighted average, rate of increase, and maximum), total lesions (measured as time-weighted average, rate of increase, and maximum), clinical observations (vital signs, weight, and signs of illness) and clinical chemistry.

Oral administration of ST-246® at 10 mg/kg/day in cynomolgus monkeys reduced disease signs (lesion number and viral DNA levels) in the first study. No variola virus-related mortality was noted following orogastric administration of ST-246® at 10 mg/kg/day or in placebo treated animals; therefore, ST-246® could not be evaluated for efficacy or prevention of mortality. The second study is ongoing and therefore the existing data are still blinded.

Progress towards approval of ST-246[®]

Dennis E Hruby

SIGA Technologies, Inc., United States of America

The drug candidate 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. To summarize its development, ST-246[®] is a highly potent, non-toxic and specific inhibitor of orthopoxvirus replication in vitro and in vivo. ST-246[®] is effective at preventing morbidity and mortality in many different animal models against a number of orthopoxviruses, including variola virus. The drug is orally bioavailable with excellent PK parameters. Final API and clinical trial material have been identified and three NDA registration batches have been completed and are currently undergoing stability testing. The drug has an open IND with Fast-Track status. It has an approved orphan drug designation in the US 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 be launched.

As ST-246[®] cannot be tested in smallpox patients, its smallpox antiviral efficacy is demonstrated using animals. In recognition of the challenges this poses, the FDA formalized in May of 2002 what has become known as the “Animal Rule” in CFR, Title 21, Volume 5, part 314.600. The central tenet of this statute stated that “FDA may grant marketing approval for a new drug product for which safety has been established and for which the requirements of 314.600 are met based on adequate and well-controlled animal studies when the results of those animal studies establish that the drug product is reasonably likely to produce clinical benefit in humans.”

Animal efficacy has been demonstrated in more than 40 animal trials using 6 animal species and 6 orthopoxvirus pathogens, in which ST-246[®] has consistently demonstrated complete protection of infected animals to morbidity and mortality. In the best characterized IV MPX:NHP model, ST-246[®] lowers viral load, reduces lesion formation, and protects from death, even when therapy is delayed until after lesion formation in infected animals. Similar results have been obtained in the variola:NHP model, despite the limitations of the model. A minimum protective dose in the NHP has been determined that suggest the proposed human dose provides at least a 3-fold safety margin. Furthermore, there have been no treatment failures due to the emergence of resistant variants in infected animals, including NHP. It is strongly anticipated that the data already collected as well as those data proposed to be collected should be sufficient to support the concept that ST-246[®] is more than reasonably likely to show clinical benefit, thereby allowing the regulatory approval of this product under CFR, Title 21, Volume 5, part 314.600.

Smallpox non-human-primate model refinement

David Ulaeto

Defence Science & Technology Laboratory, United Kingdom

The US Centers for Disease Control and Prevention (CDC) commissioned an international panel to review the variola non-human primate challenge model of human smallpox. A significant amount of information was presented: on clinical and pathologic descriptions and studies of human smallpox and monkeypox; non-human primate animal model development using intravenous administration of variola or monkeypox as challenge viruses; comparisons of the pathology observed in these studies with historic pathologic descriptions of human smallpox; non-human primate animal model development using aerosol administration of variola or monkeypox as challenge viruses; additional non-human primate animal model development of intranasal, intratracheal, intrabronchial or aerosol challenge models with monkeypox virus.

CDC asked for a review of this material and consideration of two major questions: how can “smallpox” animal model development (focusing on non-human primate studies) be improved to 1) better resemble human smallpox and/or 2) evaluate therapeutic interventions.

These considerations included, but were not limited to: route of challenge, strain or species of virus, preparation of virus, challenge dose of virus, strain and origin of animals, health/age/weight-assessments/measurements of animal species, and disease outcome measurements. An overall recommendation of the panel was the desirability of a model that both extended the asymptomatic incubation period and minimized the challenge dose of virus to provide reproducible and measurable disease outcomes.

Digitization of the smallpox eradication programme archives

Marie Villemin Partow

World Health Organization

The digitization project for the Smallpox Eradication Programme Archives started in June 2009. As of December 2010, all digitized smallpox archives had been uploaded into the dedicated database.

Smallpox Eradication Programme Archives:

Physical Fonds:

Storage :	Archives, WHO HQ
Size :	122 linear metres, 600 archival boxes, 2000 files
Date range:	1948-1987, mainly 1965-1980
Research tools:	Archives of the Smallpox Eradication Programme, a Guide and Inventory, Vol. I and II, 1982 and 1988

Digitized Fonds:

Digitization:	Colour, 1/1, 300 dpi, OCR
Format :	TIFF and PDF/A
Size :	10 TB
Storage:	SATA2 discs on MD3000i, security copies on magnetic tracks LTO3 (EMC Networker) Description and PDF/A files for consultation integrated into ERMS Livelink Enterprise Server.

Project Phases

Needs analysis:	May–September 2009
Digitization:	September 2009–August 2010
Integration into the database:	December 2010

The main challenge was the volume of paper and electronic data to manage. This was the first large-scale digitization project within the WHO Archives, so a wider perspective was taken into consideration in order to plan and re-use the knowledge and experience gained for upcoming digitization projects. The project has shown that good planning and a detailed needs analysis are mandatory and that external partners such as health technical unit scientists and IT experts are critical for success.

The main scope was the preservation of the paper files and the integration of the scanned archives into a dedicated database with a powerful search engine. Both objectives were achieved as of December 2010.

In early 2011, all WHO staff (HQ and Regions) were provided with the ability, via a SharePoint interface, to search and download files from the entire database.

External researchers and a variety of staff from academia can request access to the smallpox digitized files. There is now an urgent need to make all data available worldwide via a dedicated web interface. To achieve this, a new project is required, supported by the necessary financial and human resources.

Variola virus repositories biosafety inspection visits in 2012

Nicoletta Previsani

World Health Organization

There are currently two WHO Collaborating Centres serving as repositories for work with and storage of variola virus; one situated at the Centers for Disease Control and Prevention (CDC) in Atlanta, USA and the other at VECTOR in Novosibirsk, Russia. The two laboratories continue to work with variola virus under a process overseen by WHO, in which the work programme and other activities surrounding the work are monitored and approved by a WHO advisory committee. A number of World Health Assembly resolutions have been passed that concern the controls and assurances required by the international community for the safe and secure storage and working arrangements that is required of the repositories. Most recently, the WHA passed resolution WHA60.1 (18 May 2007) which stated that the biennial inspections of the two repositories should ‘meet the highest requirements for biosafety and biosecurity’. Following this resolution, the process in place for conducting the inspections was re-examined, to ensure that the repositories were indeed meeting the highest requirements for biosafety and biosecurity.

The publication of CWA 15793 (2008;) Laboratory Biorisk Management Standard provided an opportunity to adopt an assessment approach in line with what is regarded by many as a new benchmark for biosafety and biosecurity (collectively addressed through biorisk management). It was therefore agreed that the two inspections in 2009 would be conducted using CWA 15793 as the basis.

Prior to the visits, a protocol was drafted based upon CWA 15793, and two separate briefing meetings were held with representatives of the two repositories to explain the approach and review the content of the protocol which would be used. Inspection visits took place in May and December 2009 in the USA and Russia respectively, and the results have since been published by WHO. Both repositories positively embraced the new approach and expressed a desire to work towards an assessment mechanism which provides a high degree of transparency and structure. The revised approach was considered successful, although it is accepted and agreed that the inspection process can be further improved to the benefit of WHO, the repositories themselves and the wider community.

As a next step, WHO now plans to convene a meeting between the two repositories and biorisk management experts in late January 2011 to further refine the inspection process in view of the upcoming visits, scheduled to take place in 2012. With the involvement of dedicated personnel on all sides, it is believed that a robust approach will continue to evolve allowing effective inspections of the repositories, helping to provide an assurance to the wider community that this vital work is being conducted safely and securely, in line with the highest standards of biosafety and biosecurity.

Annex 2. Agenda of the meeting

13th Meeting of the WHO Advisory Committee on Variola Virus Research, from 31 October to 1 November 2011 Salle A, WHO, Geneva, Switzerland

Agenda

31st October 2011

9:00 – 9:15	Opening –Dr K. Fukuda, Assistant Director-General for Health Security and Environment Election of chair & rapporteur
Variola virus reports	
9:15 – 9:30	Report from the WHO Secretariat - P. Formenty
9:30 – 9:45	Update on research proposals submitted to WHO in 2011 – R. Drillien
9:45 – 9:50	Update on non-infectious DNA clones of the variola virus held at National Institute of Communicable Diseases, South Africa –R. Swanepoel
9:50 – 10:00	Comments on the variola DNA for a control variola assay evaluation protocol – I. Damon
10:00 – 10:15	Report on the variola virus collection at the WHO Collaborating Centre Repository in VECTOR, Koltsovo, Novosibirsk, Russian Federation – S. Shchelkunov
10:15 – 10:30	Report on the variola virus collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA – I. Damon
10:30 – 11:00	Tea/Coffee Break

Variola virus research 2010-2011 Update

11:00 – 11:20	Review of protein-based diagnostic development – K. Karem
11:20 – 11:40	Use of live variola virus to evaluate antiviral agents – V. Olson
11:40 – 12:00	Multiplex PCR assay for simultaneous identification of variola virus and other human pathogenic orthopoxvirus species – S. Shchelkunov
12:00 – 12:20	Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of third-generation vaccines – V. Olson

12:30 – 14:00	Lunch
---------------	--------------

- 14:00 – 14:20 Progressive vaccinia and eczema vaccinatum: insights from the laboratory
– I. Damon
- 14:20 – 14:40 Efficacy study of chemically synthesized compounds against orthopoxviruses
– A. Sergeev
- 14:40 – 15:00 PCR-based diagnostic assay for the multiplex detection of variola virus and
agents of viral haemorrhagic fever – L. Golightly
- 15:00 – 15:20 FDA's efforts to facilitate development and approval for smallpox medical
countermeasures – L. Borio

15:20 – 15:50	Tea/Coffee Break
---------------	-------------------------

- 16:00 – 16:20 Progress on the development of the smallpox vaccine IMVAMUNE®
– P. Chaplin
- 16:20 – 16:40 Update on LC16m8 vaccine - H. Yokote
- 16:40 – 17:00 Update on development of hexadecyloxypropylcidofovir (CMX001)
– R. Lanier
- 17:00 – 17:20 Progress and challenges on looking at ST-246 in treatment of variola
challenged non-human primates – A.J. Goff
- 17:20 – 17:40 Progress towards approval of ST-246 – D. Hruby
- 17:40 – 18:00 Smallpox non-human-primate model refinement – D. Ulaeto
- 18:00 – 18:10 Additional presentation – K. Karem
- 18:10 – 18:30 General discussion

18:30 – 19:30	Social event
---------------	---------------------

DAY ONE CLOSES

**13th Meeting of the WHO Advisory Committee on Variola
Virus Research, from 31 October to 1 November 2011
Salle A, WHO, Geneva, Switzerland**

1st November 2011

9:00 - 9:15 WHO smallpox vaccines: update – P. Formenty
9:15 – 9:30 Digitization of the smallpox eradication programme archives – M. Villemin
9:30 – 9:45 Variola virus repositories biosafety inspection visits 2012 – N. Previsani
9:45 – 10:00 Renewal of the scientific subcommittee for the next 3 years – WHO secretariat
10:00 – 10:15 Outline key scientific and programmatic agenda for the next 3 years
 General discussion and preparation of draft meeting report

10:15 – 10:45 Tea/Coffee Break

10:45 – 12:00 General discussion and preparation of draft meeting report (continued)

12:00 – 13:30 Lun ch

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 meeting report

ACVVR MEETING CLOSES

Annex 3. List of participants

13th Meeting of the WHO Advisory Committee on Variola Virus Research from 31 October to 1 November 2011, Salle A, WHO Headquarters, Geneva

LIST OF PARTICIPANTS

MEMBERS OF THE ADVISORY COMMITTEE

Dr Isao Ari ta, 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 Ali Sha n Khan, Deputy Director, National Center for Zoonotic, Centers for Disease Control and Prevention Vector-Borne and Enteric Disease, Atlanta GA, UNITED STATES OF AMERICA

Dr George W. Korch, Acting Principal Deputy Assistant Secretary for Preparedness & Response, Department of Health and Human Services, Washington, D.C., UNITED STATES OF AMERICA

Professor J. Michael Lane, MD MPH, 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 Brazzaville, CONGO

Dr Andreas Nitsche, Highly Pathogenic Viruses, Centre for Biological Safety, 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 Head, 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 M uyembe T amfum*, Director, Institut National de Recherche Bio-Médicale (INRB) Kinshasa, DEMOCRATIC REPUBLIC OF THE CONGO

Dr Oyewale Tomori, Regional Virologist, 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, Vice President, Maharashtra Association for the Cultivation of Science Pune, INDIA

Dr Luciana L. Borio, Acting Assistant Commissioner for Counterterrorism Policy 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 (CDC) Atlanta, UNITED STATES OF AMERICA

Dr Linnie Golightly, Associate Professor of Clinical Medicine and Microbiology and Immunology, Weill Medical College of Cornell University, Division of Infectious Diseases, New York, NY, 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

Dr Peter Jahrling, Director, National Integrated Research Faculty, National Institute of Allergy and Infectious Diseases, Bethesda MD, UNITED STATES OF AMERICA

Dr Kevin Karem, Acting Team leader of the Poxvirus Team, Centers for Disease Control and Prevention (CDC), Atlanta GA, UNITED STATES OF AMERICA

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

Professor Hermann Meyer, Head of BSL-3 Laboratory, Bundeswehr Institute of Microbiology, Lohhof, GERMANY

Dr Shigeru Morikawa, Chief, Laboratory of Special Pathogens, Department of Virology 1, National Institute of Infectious Diseases, Tokyo, JAPAN

Dr Bernard Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda MD, UNITED STATES OF AMERICA

Dr Victoria Olson, Research Microbiologist, Poxvirus Program, Division of Viral and Rickettsia Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, UNITED STATES OF AMERICA

Dr Jean-Claude Piffaretti, Interlifescience, Massagone, SWITZERLAND

Dr Robin Ruepp, Product Team Leader, Safe and Efficacy of Medicines, European Medicines Agency (EMA), London, UNITED KINGDOM

Dr Artemiy Sergeev, Chief, Laboratory of Variola Virus and other Orthopoxvirus Infections FBRI 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 Sergei Shchelkunov, Head, Department of Molecular Biology of Genomes, State Research Center of Virology and Biotechnology VECTOR, Federal Service for Surveillance on Consumer Rights Protection and Human Well-being, Koltsovo, Novosibirsk region, RUSSIAN FEDERATION

Dr David Ulaeto, Scientific Leader Biomedical Sciences DERA-CBD, Salisbury, Wiltshire, UNITED KINGDOM

INVITEE PRESENTERS

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

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

OBSERVERS

Dr Ichiro Kurane, Deputy Director General, National Institute of Infectious Diseases (NIID), Tokyo, JAPAN

Mr Vladimir Ryabenko*, Head, Department of International Relations, FBRI 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

WORLD HEALTH ORGANIZATION

REGIONAL OFFICES

Dr Francis Kasolo, Programme manager, Integrated Disease Surveillance Programme,
Representative for AFRO

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

Dr Hassan El Bushra*, Representative for EMRO

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

Representative for SEARO*

Representative for WPRO*

HEADQUARTERS

Dr Keiji Fukuda, ADG/HSE

Dr Marie-Paule Kieny*, ADG/IER

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

Dr Joachim M. Hombach*, Director, FCH/GAR/IVR a. i

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

Mr Gian Luca Burci*, Legal Counsel, DGO/DGD/LEG,

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

Dr Cathy Roth, Adviser, HSE/HEA

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

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

Dr Margaret Lamunu, HSE/GAR/BDP/EDP

Dr Pierre Formenty, Coordinator, HSE/GAR/BDP a.i.

Mr David Bramley, HSE/PED, Consultant, (Rapporteur)

* Unable to attend