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WHO Advisory Committee on Variola Virus Research

Report of the Seventh Meeting

Geneva, Switzerland
10 –11 November 2005

EPIDEMIC AND PANDEMIC
ALERT AND RESPONSE

World Health Organization

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Contents

1. Introduction and report of the Secretariat

1.1 Dr Mike Ryan welcomed participants to the seventh meeting of the Advisory Committee on Variola Virus Research and indicated that the WHA is showing an increased interest in the topics to be discussed. The Director General of WHO had made it clear that biosecurity and biosafety issues relating to work on live variola viruses had assumed greater significance during the past year. He has specifically requested additional advice from the Advisory Committee on the recommendation made at the last meeting regarding the expression of variola virus genes in other orthopoxvirus species.

1.2 Dr Cathy Roth indicated that the purpose of the meeting was to review the progress of current essential research that depended on access to live variola virus and to advise WHO on the continuing need for this research. She reiterated that this research should be output-oriented and not open-ended, open and transparent and necessary for public health. She reminded all researchers to provide the Secretariat with abstracts of the research done so their results could be disseminated to all interested parties.

1.3 Dr Daniel Lavanchy then stated that the proceedings of the Advisory Committee were confidential but that its final report would be a public document and posted on the WHO web site. He also indicated that the African Regional Office had asked for smallpox research progress, and review thereof, to be included as a special item on the Executive Board agenda and that it was possible that it might request the cessation of all further research in this area. He suggested that the Advisory Committee should take this into account during their deliberations.

1.4 Following agreement by members of the Advisory Committee, Professor Geoffrey Smith was appointed Chairman and Dr Peter Greenaway Rapporteur.

2. Update on variola virus strains in the two virus repositories

2.1 Professor Sergei Shchelkunov presented a paper describing the status of the variola virus repository held in the Russian collection at the Russian State Centre for Research on Virology and Biotechnology (VECTOR). Some 120 strains, including 17 clinical isolates, were held and tests had shown that 32 out of 55 of these were viable; it was now planned to assess the viability of the remaining strains in the collection.

2.2 Preparations of variola virus DNA were being conserved in three ways – as full length genomic DNA preparations, as extended PCR amplicons covering the full genome and as DNA fragments cloned in hybrid plasmids. All materials were associated with documentation describing derivation, method of preparation and storage. The most reliable method for the long term conservation of variola virus DNA sequences was considered to be cloning in plasmids.

2.3 In response to a question regarding the need for further work in this area, Professor Shchelkunov stated that the viability of all strains held in the collection needed to be assessed and their DNA conserved.

Action : Professor Shchelkunov to submit a proposal to the Advisory Committee describing essential research needed to support the conservation of genetic material in the variola virus strains held in the Russian collection.

2.4 The repository at the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America contains items donated from five worldwide collections. The repository contains 451 semi-independent items: samples, isolates, or strains. Of the 451 items, 238 were viruses with recorded geographical location of identification and 228 had a known date of isolation. Added to this collection, in 2005, were the remainders of scabs possibly collected from an 1882 outbreak of smallpox in Boone County, Arkansas. Attempts at isolating viable virus were unsuccessful and DNA isolated from the material was inadequate for PCR analysis nor identification of any orthopoxvirus in the material. The material is currently being stored within the liquid nitrogen freezer in the maximum containment laboratory.

2.5 Dr Olsen indicated that a duplicate set of ampoules held in a secure back-up freezer were physically inventoried in April 2005 and transferred to a modern liquid nitrogen freezer. The genomes of 47 strains had been sequenced, and 45 of these had been analysed by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) analysis. The data obtained has been used to construct phylogenetic trees; ePCR/RFLP analysis suggests the strains group into two divergent clades.

2.6 Attempts to correlate biological properties of viable strains (comet formation by extracellular enveloped virus, EEV) with virulence (as reflected by case-fatality rates in the outbreaks from which the strains were derived) were described. No overall correlation was found across all strains within the clades, but association of comet phenotype was observed across clades. This work is relevant to the in vitro assessments of the potential efficacy of vaccines against variola virus. Specific proposals to do further research on this topic were not discussed.

2.7 It was noted that both Collaborating Centres were acting as guardians of the variola virus collections and that all research must be approved in advance by the Advisory Committee. It was also noted that requests for variola virus DNA samples had been made to the CDC by a variety of researchers. However, changes in the legislative requirements within the United States of America now needed to be clarified before distribution of variola virus DNA fragments could continue under the auspices of the 1994 recommendations of the Ad Hoc Orthopoxvirus Advisory Committee. Previous requests had been fulfilled in accordance with the recommendations made previously by the above mentioned Advisory Committee. It was reported that no distribution of variola DNA by VECTOR had occurred.

2.8 The WHO secretariat reminded the Committee that all such requests must be channeled through them so that an accurate record of all groups holding variola virus DNA could be maintained. It was proposed that this information should be made available to the Advisory Committee.

Action: WHO secretariat to provide information on the distribution of variola virus sequences to the next meeting of the Advisory Committee.

2.9 Clarification of the status of the hybrid viruses held in the CDC repository was requested. Representatives from the repository indicated that i) these viruses had not been destroyed, ii) no date had been set for their destruction based on security considerations as determined by United States government officials, and iii) they had been used during the last year for assessment of new diagnostic strategies and two viruses had been sequenced. Members of the Advisory Committee were then informed that although the total number of strains held by each repository had remained constant, the actual number of experimental samples that might contain variola virus, generated through various experiments, had now increased because of work in progress. A full inventory of the "items" in the repository freezers, as well as a full accounting of the working and master seed stocks was provided to the Committee Secretariat by the WHO CC at CDC in Atlanta at the meeting.

Action: Records of the stocks, including working stocks, of all variola virus samples held by each repository and submitted to the WHO secretariat as part of their normal reporting procedures will continue to be made available to the Secretariat of the Advisory Committee at its annual meetings.

3. Update on diagnostic assays

3.1 Dr Inger Damon described recent developments in diagnostic strategies utilizing real-time PCR. These could produce definitive results within 2–4 hours, using robotics to extract samples, after submission of a clinical specimen. Assay specificities are >99%, but the predictive value positive diminishes significantly in a no or low disease prevalence situation. Thus multiple different assays will be required to confidently make the diagnosis of the first case(s) of smallpox should it re-emerge. The robustness of the tests with respect to use for vaccine adverse event monitoring, diagnostic evaluation of pustular rashes and monkeypox testing was being investigated in a number of centres. Data on sensitivity, specificity, and positive and negative predictive values were presented. Relevant information was being gathered prior to a planned submission to the United States Food and Drug Administration (FDA) for review and subsequent approval of one of the assays.

3.2 It was noted that many nucleic acid based diagnostic procedures analysed less than 0.5% of the variola virus genome. Analyses of the rabbitpox – variola virus hybrids and the cowpox-variola virus hybrids in the CDC collection had demonstrated the limitations of this approach: neither was accurately identified as including variola virus DNA using real-time PCR approaches currently in use. Extended PCR/RFLP analyses and, indeed, full length DNA sequencing strategies resolved some of these limitations. Research investigating a novel high throughput pyrosequencing methodology for DNA analysis had therefore been initiated.

3.3 The Advisory Committee asked for more to be done to ensure that there was adequate translation of the existing diagnostic technologies out into the field – often the poorly

resourced developing countries that might represent the front line for detecting an emerging smallpox outbreak.

Action: The WHO secretariat, in association with the Collaborating Centres, to develop dissemination plans for the transfer of current diagnostic technologies (including training) to both clinical centres and reference laboratories involved in smallpox surveillance, detection and rapid diagnosis. This might involve either the distribution of validated PCR detection kits or (more simply) better information on how to get suspect clinical samples transferred to one of the Collaborating Centres. This action item will require a significant use of resources, more so if the former approach is proposed.

3.4 The need for the above actions was underlined by the presentation from Dr Herman Meyer who described an evaluation of published real-time PCR assays designed to differentiate and identify variola virus from other orthopoxviruses. It was demonstrated that some published assay methods yielded false-positive amplifications with non-variola orthopoxviruses. Due to serious consequences of a misdiagnosis of smallpox, more that one assay has to be applied in order to reliably identify smallpox. Access to diagnostic procedures that were based on different and validated detection parameters were therefore considered essential.

3.5 The resulting discussion demonstrated that a number of unpublished (and non validated) diagnostic procedures had been developed in many laboratories and that these needed to be validated. This was reinforced by Professor Shchelkunov who described the development of multiplex PCR and microarray technologies for the species-specific differentiation of orthopoxviruses. Research had resulted in a number of improvements that meant that genus and species-specific poxvirus diagnosis could be done against the backdrop of coincident herpesvirus or varicella virus infections.

3.6 It was concluded that an adequate number of PCR based detection and diagnostic systems for variola virus had been developed and that no further research involving the use of live variola virus strains or hybrid viruses was necessary. A few members, who felt further research in this area was needed, especially in the development of protein-based diagnostics, challenged this view.

4. Update on antibodies

4.1 Dr Kevin Karem described the development of IgM based ELISA tests for the detection of human orthopoxvirus infections. This work had been initiated as a way of standardizing assays for use during a monkeypox outbreak in humans and as a way of monitoring the infection of monkeys challenged with variola virus. He emphasized that, in the absence of samples that could be used for PCR analyses, serology (and IgM ELISA) might be the only way to carry out the surveillance of poxvirus infections to generate incidence rather than prevalence data.

4.2 Using samples obtained from the human monkeypox outbreaks in the USA (2003) and in the Republic of Congo, Dr Karem reported that IgM serologic testing could be used as a diagnostic support for acute infection. However, it could not be considered as a stand

alone diagnostic assay. Retrospective analyses indicated that the IgM ELISA provided up to 95% specificity and 95% sensitivity for diagnosing acute monkeypox infection.

4.3 Dr Karem argued that IgM ELISA procedures had utility in providing diagnostic support for orthopoxvirus infections, especially those involving a non-specific rash. In addition, the detection of IgM in monkeypox patients previously vaccinated against smallpox provides evidence for the use of the assay in vaccinated populations exposed to heterologous orthopoxviruses. The Committee acknowledged that the development of these monkeypox ELISA reagents was most relevant for Africa and had been dependent on support being given to the variola virus research programme.

4.4 Dr Nina Tikunova then described her work on the generation of fully human anti-orthopoxvirus monoclonal antibodies using phage display technology. This necessitated the generation of a new phage-display library of human single chain Fv antibodies from Vh and VI genes cloned from the peripheral lymphocytes of vaccinia virus immunized donors. The resulting library size was calculated to be more than 3×10^7 and its quality was verified by determining the percentage of clones with an appropriately sized insert.

4.5 This library was subsequently panned against vaccinia virus and 8 unique antibodies were identified. Of these, 4 were neutralizing antibodies and were shown to recognize a 32-kDa protein. Further work leading to the construction and characterization of full sized human monoclonal antibodies was then done. After purification by affinity chromatography, the ELISA and neutralization assays were repeated and Western blot analysis was used to identify the target proteins.

5. Sequence analysis of variola virus DNA

5.1 Professor Shchelkunov described further work on the construction of phylogenetic trees of variola viruses using, in particular, the gene encoding α/β interferon binding protein. It was noted that cowpox virus strains gave a heterogeneous distribution pattern across the constructed dendograms, whereas variola virus strains were much less divergent.

5.2 This work augmented that of previous years and added to the information base available on the evolutionary history of the variola viruses. It was agreed that sufficient sequence information had now been obtained.

6. Biological properties of variola virus strains

6.1 Dr Elena Ryabchhikova then described her work on the comparison of extracellular enveloped virus (EEV) produced by different strains of variola virus. She noted that the pattern of assembly and maturation was identical for the India 3A, Congo 9 and Butler strains in different cell types. Her work focused on the production of EEV and she observed that the ability to produce large amounts of EEV did not correlate with virulence. It was agreed that understanding the mechanisms involved in the assembly of virus particles could facilitate the development of new anti-viral compounds.

7. Update on animal models

7.1 Dr Peter Jahrling then described further refinements to the primate model of human smallpox; the goal of this work is to better develop the model for future application in antiviral drug efficacy evaluation and ultimate "registration" under the FDA animal efficacy rule. This work is done in collaboration with scientific counterparts at the CDC; all work with live variola virus is done within the maximum containment laboratory using protocols approved by various biosafety committees. Specifically, a sequential sacrifice study was performed using two groups of monkeys receiving doses of variola virus calibrated to cause either 30% mortality (similar to common smallpox in humans) or 100% mortality (similar to haemorrhagic or "black" smallpox). These studies were designed to better characterize the progression of disease patho-physiology, in compliance with the FDA Animal Efficacy Rule. Complete necropsies were performed on monkeys sacrificed at frequent intervals over the disease course to provide tissues for examination by quantitative virology, immuno-histopathology, flow cytometry, and cytokine ELISA tests.

7.2 The distribution of viral antigens demonstrated by immuno-histochemistry as well as replicating virus in electron micrographs correlated with pathology in lymphoid tissues, skin, oral mucosa, gastrointestinal tract, reproductive system, and liver at all intermediate and terminal time points evaluated. Apoptosis was a prominent observation in lymphoid tissues with a striking loss of T cells observed, probably a consequence of pro-inflammatory cytokines. The detection of fibrin degradation products as well as other changes in haematology in monkeys that developed classical or haemorrhagic smallpox suggests that activation of the coagulation cascade is a component of both disease syndromes.

7.3 Disease progression in both the lesional (low dose, common smallpox) and haemorrhagic (high dose) models, as demonstrated by gross and histological examination, did not appear until several days after significant virus replication had occurred, as judged by quantitative PCR. In the lesional model, on day 3, skin lesions were present on about half of the animals as macules or papules. By day 5 cutaneous lesions were extensive, averaging 695±91 lesions per animal. Testicular haemorrhage, lymphadenopathy, splenomegaly, mild to marked splenic lymphoid depletion, lymphoid depletion of gut-associated lymphoid tissue and hyperplasia of the non-proliferating pool of the myeloid cells were predominant features.

7.4 By day 7 all animals had cutaneous disease (average 719± 137 lesions), most severe on the legs, arms and head with some areas where lesions were nearly confluent. The intensity of viral antigens in tissues continued to increase. By day 9 skin lesions, often with epithelial hyperplasia, were present mainly as crusts and scabs but a few oral lesions remained. By day 11 most of the skin lesions were either crusts and scabs or had been resolved.

7.5 In the 100% lethal, haemorrhagic model, viraemia titres were 3 logs higher than in the lesional model, when tested at 24 hours post infection. Titres in bone marrow increased from 5 x 10^9 genomes/gm on day 1 to 10^{10} to 10^{11} genomes/gm on day 3, spleen levels increased from 10^{10} genomes/gm on day 1 to 5 x 10^{13} in one monkey on day 2 and typically to 5 x 10^{11}, and liver from 10^9 to 10^{12} genomes/gm. Virus titres in other

tissues peaked on day 2 to 3 with levels from 10^5 to 10^{10}; all animals died between days 3–4 post infection.

7.6 Dr Jahrling indicated that these sequential sacrifice studies help to inform the interpretation of cidofovir treatment studies using the model. Viraemia titres alone underestimate the extent of systemic infection at the time cidofovir treatment was successfully initiated (24 or 48 hours post infection). At 24 hours post infection, virus replication in spleen exceeded 10^9 genomes/g, while bone marrow and liver had approximately 10^8 genomes/g. These appear to be the initial sites of replication, because at 24 hours post infection other tissue had levels between 10^5 and 10^6 but blood had only 10^4 genomes/ml. When "pox lesions" appeared they had virus levels equal to midcourse bone marrow and blood. As poxvirus lesions increased in both number and size they became a major site of virus replication.

7.7 It was acknowledged that the current animal model still needed some refinement and that alternative routes of infection, possibly using the intra-tracheal route, might be explored. Advice from the FDA was being sought on which clinical parameters should be made available to support future Investigational New Drug Application (IND) submissions. It was noted that there would be different requirements for the evaluation of new vaccines and antiviral compounds. Generally, the evaluation of second generation vaccines would not require the use of a variola based animal model as non-inferiority to licensed smallpox vaccines could be used. In addition, third generation vaccines will be evaluated using surrogate animal models under the FDA animal rules. These circumstances do not apply during the licensure of new anti-viral compounds, for which direct activity against variola virus must be demonstrated and a variola virus-based animal model may be required.

7.8 The main objective of this work is to build bridges between the monkeypox and variola virus animal models in order to facilitate antiviral drug development and subsequent licensure. The Committee agreed that this work is essential but that new research proposals were required describing exactly what research was proposed. These proposals should address issues associated with biosafety and biosecurity.

Action: Dr Jahrling and collaborators to submit updated proposals to the Advisory Committee for future work aimed at developing a suitable non-human primate animal model of human smallpox.

8. Update on vaccines

8.1 Professor Alexander Sergeev reported a study of the reactogenicity, safety and immunogenicity of a recombinant smallpox and hepatitis B bivalent vaccine for oral administration in humans. Among the different protocols tested, double administration of the bivalent vaccine to volunteers (at a lower and a higher dose) at different intervals between the administrations (1–2 weeks) caused only minor local and general reactions. Significant levels of anti-vaccinia virus antibodies were induced in 90 to 100% of the volunteers and these responses lasted for more than 6 months. Satisfactory levels of anti-hepatitis B antibodies were not detected.

8.2 Dr. Stittelaar presented experiments undertaken to compare the efficacy of post exposure smallpox vaccination versus antiviral treatment with acyclic nucleotides against monkeypox virus infection. A lethal dose of monkeypox virus was administered to a group of monkeys some of which were vaccinated intradermally one day later with the vaccinia virus Elstree RIVM strain while others were treated daily either with cidofovir or with HPMPO-DAPy. Vaccination failed to prevent progression of the disease in all animals except one. Both antiviral drugs provided a significant although not complete protection against the disease. Future studies are being planned to assess the potential of combining vaccination and antiviral treatment under a post-exposure situation.

8.3 Presentations were then given by representatives of three different companies involved in the development of 2nd and 3rd generation smallpox vaccines.

- Dr. Bedford from Acambis reported clinical trials using ACAM 2000, a cloned vaccinia virus isolated from the Dryvax vaccine (vaccinia virus NYCBH). The particular clone retained for testing in volunteers had shown good immunogenicity and low neuro-virulence in mice. When used in primary human vaccination trials it gave a high level of takes (99%) and most often induced neutralizing antibodies (96%) to vaccinia virus. When used as a booster vaccine it induced takes with a lower frequency than Dryvax but the rate of sero-conversion was similar. No significant difference was noted in the frequency of adverse events caused by ACAM 2000 and Dryvax. Dr. Bedford also reported a phase 1 study using the modified vaccinia virus Ankara strain designated as ACAM 3000. This highly attenuated vaccinia virus strain was thought to be safer for the vaccination of immunocompromised individuals and for those with skin conditions. Vaccination of 110 volunteers was well tolerated and no adverse events were noted. ELISA and neutralizing antibody titres against vaccinia virus were found in more volunteers after two inoculations than after only one. A phase 2 trial is currently planned.

- Dr. Gordon from Vaxgen described the attenuated LC16m8 strain derived from the Lister strain. This strain had been licensed in Japan in 1975 before the eradication of smallpox. It was shown to be less virulent in children than the parental Lister strain according to EEG analysis as well as less neuro-virulent in monkeys. A recent clinical trial performed on 153 volunteers has given a 100% take and sero-conversion rate. Anti-vaccinia antibodies were induced that were reported to neutralize both intracellular mature virus (IMV) and extracellular enveloped virus (EEV) despite truncation of the B5R gene encoding a major target of EEV neutralization. Clinical trials with this vaccine are ongoing.

- Dr. Chaplin from Bavarian Nordic described studies carried out with the modified vaccinia virus Ankara strain MVA-BN. A brief review of the derivation of this strain and its avirulent phenotype in severely immunocompromised mice was presented. MVA-BN was shown to provide protection against monkeypox in monkeys if vaccination was performed only 3 days before challenge but not if vaccination was performed on the same day as challenge. Six clinical studies have been completed with this strain and no adverse events have been reported after vaccination of more than 500 volunteers with HIV or atopic dermatitis. A single vaccination induced sero-conversion in

94% of the volunteers whereas two injections were required to achieve 100% sero-conversion.

8.4 It was noted that new information was being gained on the safety and efficacy of 2nd and 3rd generation smallpox vaccines and several companies were gearing up for the manufacturing of large amounts of these vaccines. Some members of the Committee expressed concern about the possible lack of worldwide availability of the new vaccines. This was considered outside the mandate of this Committee but will be dealt with during the discussion of the WHO proposals for the stockpiling of smallpox vaccines. Finally, it was considered that there was no scientific need or regulatory requirement for assessing smallpox vaccines in animal models using live variola virus.

9. Review of candidate antiviral drugs

9.1 Dr John Huggins reviewed the properties of the non-human primate animal models available for assessing candidate antiviral drugs particularly with respect to their ability to meet the regulatory requirements likely to be required by the FDA. He noted that the development of safe and effective antiviral drugs that would reduce mortality is critical for improving public confidence and reducing the impact of bioterrorist threats.

9.2 Dr Huggins then presented data on the properties of a derivative of cidofovir that could be delivered orally. Drug uptake was through the normal lipid uptake pathway and this led to its rapid and widespread distribution in treated animals. However, it was noted that the drug was partially metabolized by the cytochrome P450 pathway. Co-administration of ritonavir was shown to prevent this metabolism and to increase bioavailability

9.3 Dr Huggins indicated that proposed future work would focus on studying the efficacy of intravenously administered cidofovir in both the monkeypox and smallpox lesional models using GLP protocols. During discussion it was noted that anti-viral drugs would be most beneficial if they could be given following disease onset rather than only shortly after exposure. It was also noted that the requirements of regulatory agencies other than the FDA may need to be considered when suitable antiviral drugs became available for licensing.

9.4 Dr Evgueny Belanov then described his work on the development and assessment of a number of candidate antiviral compounds based on 1,2,4-triazine derivatives. He used these to generate drug-resistant mutants of vaccinia virus and to demonstrate that the drugs identified have different targets within the virus. No information was presented on the toxicity of the identified drugs. This, and the identification of the drug targets, would be the focus of his proposed future work.

9.5 Dr Inger Damon described an alternative approach to the development of antiviral drug therapies for smallpox infections. This followed on from the presentation made by Dr Kalman last year. The proposed approach involved targeting protein kinases that play a role in host signaling pathways. The proposed approach involved modifying host signaling pathways using small molecules. The example of tyrosine kinase systems was discussed. Three separate compounds, in various stages of

clinical trial development, were assessed that inhibited different tyrosine kinases. These produced effective restriction of virus release. She indicated that future work would involve other orthopoxviruses with a view to further understanding the mechanism of action and cell biology of the drugs.

9.6 Dr Hruby presented data on the discovery and development of ST-246, a candidate smallpox antiviral drug. In tissue culture the drug is 8000 times more potent than cidofovir and it can be delivered orally. It is effective against all orthopoxviruses tested. In the presence of drug, vaccinia forms normal amounts of intracellular mature virus but the envelopment of this virus to form intracellular enveloped virus (IEV) and the subsequent dispersal of virus and plaque formation was inhibited. The molecular target for ST-246 is protein F13.

9.7 ST-246 gave solid protection in the ectromelia virus mouse model. It was also shown to be highly effective when given 4– 2 hours post infection and when the duration of treatment was longer than 7 days. Protection was also demonstrated in the monkeypoxvirus/ground squirrel model. Preclinical, toxicology and clinical trials were now being planned.

10. Miscellaneous

10.1 The exchange of variola virus specimens between the Collaborating Centres was discussed. This debate was initiated because the 1994 report of the Ad Hoc Committee on Orthopoxvirus Infections recommended that each repository should hold a duplicate set of archived material. After consultation with members of the Ad Hoc Committee, the WHO secretariat confirmed that the recommendation was concerned with the exchange of cloned DNA only. It did not refer to the exchange of live virus samples, which would not be permitted. The main purpose of this recommendation was to ensure that precious material was not lost. Hence the intention was that each Collaborating Centre should duplicate its archived stock and locate this in a separate back-up storage facility within the Centre. The recommendation was not advocating the exchange of DNA samples between the Collaborating Centres.

10.2 The use of Standard Operating Procedures (SOPs) and a standard set of strains of variola virus by the Collaborating Centres for the assessment of candidate vaccines and antiviral compounds were discussed. All agreed that the development and use of SOPs was essential. It was also agreed that the use of a standard set of strains was desirable. It was noted that the Butler and Congo-9 strains were already designated as standard strains but that the use of an additional pathogenic Asian variola virus strain would be useful. A concern was raised that the "standard" strains were, generally passaged multiple times – and the specific passage histories of isolates at the two Centers may not be the same. A common Asian variola virus strain is not present in each repository and so some discussion is necessary to select which strains should be used.

Action: The two Collaborating Centres should maintain SOPs and should again attempt to identify reference variola virus strains (preferably already held in both centres) for use in assessing new antiviral compounds. The Centres should ascertain whether antiviral screening SOPs are still comparable.

10.3 It was strongly recommended that specific proposals for essential work using live variola virus should be submitted to the Advisory Committee. It was agreed that this should apply to all current work as it was now some five years since all the original proposals had been reviewed. It was considered likely that these would need refinement and re-focus in the light of the progress made. It was therefore proposed that all research on live variola virus would require submission of new proposals to the Advisory Committee by 31 January 2006. Approval of specific proposals was needed for work with live variola virus to continue beyond 1 May 2006. These proposals (up to 2 pages) should describe research that is essential, specific, focused and, importantly, time limited.

10.4 There was considerable discussion of this proposal and particularly the timescale involved. However, it was noted that the Scientific Sub-Committee could take decisions very rapidly, particularly if the proposals were dealt with electronically. There could be an appeal process that would probably mean that any failed proposal was considered by the full Advisory Committee (probably electronically). Members of the Advisory Committee were concerned that this procedure was not seen as a road block to research but rather, as a means of transparently demonstrating that the Committee was providing appropriate oversight of all research using live variola virus. A request was made to reassess the Sub-Committee membership.

Action: WHO secretariat to identify a format by 31 December 2005 for the proposals and establish a protocol and timescale for submission of new proposals for essential research involving live variola virus.

10.5 There was then a brief discussion on the new International Health Regulations. These require each country to establish a core capacity to undertake the diagnosis of infectious diseases such as smallpox at a level that is commensurate with the resources available. The Committee recognized that different levels of diagnostic certainty would be required in different situations. Index cases of any re-emerged smallpox infection would almost certainly require confirmation by sequence analysis at one of the Collaborating Centres. The diagnostic capacity at national level would probably need confirmation using PCR technologies. It was considered essential that each country should have suitable training programmes in place to support smallpox diagnosis. The Advisory Committee encouraged those laboratories that had developed validated diagnostic tests for smallpox to disseminate their findings and make validated reagents widely available.

10.6 Finally, at the request of the Director General, the Advisory Committee reconsidered the recommendation made at its last meeting that the expression of natural or engineered variola virus genes in orthopoxvirus vectors might be permitted if:

- the research protocols and risk assessments are reviewed for biosafety and recombinant DNA concerns and approved by appropriate institutional authorities and the WHO Advisory Committee on Variola Virus Research in accordance with national regulations and WHO resolutions and recommendations;

- those generating and handling such recombinant viruses should have their smallpox vaccination status approved by their national and institutional authorities;

- not more than one variola virus gene is inserted into the virus vector. Any proposal to insert more than one variola virus gene into an orthopoxvirus must be considered by the WHO Advisory Committee on Variola Virus research;

- the experiments are performed at BSL-3 or higher containment and consideration is given to the use of high-efficiency particulate air (HEPA) filtration of exhausted air as an additional biosafety requirement for these laboratories;

- work with such recombinant viruses is done in a laboratory in which no other orthopoxvirus is present.

10.7 The Director General had raised concerns about this recommendation because of the biosafety and biosecurity issues involved. The Advisory Committee was divided on whether to retain this recommendation and it therefore failed to reach a consensus view. Arguments were made that the use of such recombinants could be justified scientifically. The view was expressed that the issues of biosafety could be addressed by including further restrictions on the work and by using highly attenuated orthopoxviruses. It was also re-iterated that all such proposals must be considered by the Committee on a case-by-case basis. However, the Committee also recognized that the issue was a politically sensitive one and that it could lead to a loss in public confidence in smallpox research. On balance, it was therefore decided that it would be expedient to withdraw the recommendation in its entirety.

Annex 1. Agenda

10 November 2005

9:00 – 9:15 Welcome, purpose of the meeting

9:15 – 9:30 Report of the secretariat – D. Lavanchy

9:30 – 10:00 Update on variola virus strains in collection

- "An update on variola virus stocks. Conservation of genetic material of different variola virus strains from the Russian collection"
  - L. Sandakhchiev/S. Shchelkunov ("VECTOR" – Russian Federation)
- "Update on the variola strains at the WHO Collaborating Centre"
  - V. Olson (CDC – USA)
- Discussion on distribution of DNA materials upon request.

10:00 – 10:30 Update on diagnostic assays

- "Update on diagnostic development"
  - I. Damon (CDC – USA)
- "Evaluation of published real-time PCR assays, designed to differentiate and identify variola virus, using a panel of non-variola orthopoxviruses"
  - H. Meyer (Institute of Microbiology of the Bundeswehr – Germany)

10:30 – 11:00 Tea/coffee break

11:00 – 11:30 Update on diagnostic assays (continued)

- "Development of modern methods for species-specific differentiation of orthopoxviruses"
  - L. Sandakhchiev/S. Shchelkunov ("VECTOR"–Russian Federation)
- "Human Orthopoxvirus infection detected by IgM ELISA"
  - Kevin L. Karem (CDC – USA)

11:30 – 11:45 Update on antibodies

- "Generation of anti-orthopoxvirus fully human Mabs derived from phage display single-chain antibodies"
  - N. Tikunova ("VECTOR"– Russian Federation)

11:45 – 12:00 Sequence analysis of variola virus DNA

- "Viral DNA sequence analysis"
  - L. Sandakhchiev/S. Shchelkunov ("VECTOR" – Russian Federation)

12:00 – 12:15 Biological properties of variola virus strains

- "Comparative study of extracellular enveloped virus formation by different variola virus strains"
  - E. Ryabchikova ("VECTOR"– Russian Federation)

12:15 – 12:45 Update on animal models

- "Refinement of the primate models for variola"
  - P. Jahrling (NIH/NIAID – USA)

12:45 – 13:30 Lunch

13:30 – 15:15 Update on vaccines

- "The study of reactogenicity, safety and immunogenicity of recombinant smallpox- and hepatitis B bivalent vaccine for oral administration in humans"
  - A. Sergeev ("VECTOR"– Russian Federation)
- "Comparison of the efficacy of post exposure smallpox vaccination versus antiviral treatment with acyclic nucleotides against monkeypox virus infection"
  - K. J Stittelaar (Erasmus Medical Center – The Netherlands)
- 15 min presentations by:
  - P. Bedford, Acambis
  - L. Gordon, Vaxgen
  - H. C. Teisen, Bavarian-Nordic

15:15 – 15:45 Tea/coffee break

15:45 – 17:15 Review of antiviral candidate drugs

- "Update on antivirals for treatment of smallpox "
  - J. Huggins (USAMRIID - USA)
- "An update on the results of search for antiviral compounds for treatment and prevention of orthopoxviral infections"
  - E. Belanov ("VECTOR"– Russian Federation)
- "Small molecule inhibitors/tyrosine kinase inhibitors: effects on variola virus"
  - I. Damon (CDC – USA)
- "ST-246: discovery and development of a smallpox antiviral drug candidate"
  - D. Hruby (Oregon State University - USA)

17:30 – 19:00 Social event at the WHO main cafeteria

11 November 2005

09:00 – 10:00 Miscellaneous

- Exchange of variola virus specimens between the Collaborating Centres
  - request of L. Sandakhchiev
- Submission of research proposals
- Re-examination of the 2004 proposal of the advisory committee to permit the inclusion of smallpox virus genes into other orthopox viruses
  - request by WHO Director General for reconsideration

10:00 – 10:30 General discussion and preparation of draft recommendations

10:30 – 11:00 Tea/coffee break

11:00 – 12:30 General discussion and preparation of draft recommendations (continued)

12:30 – 13:30 Lunch

13:30 – 15:00 Consensus on recommendations

MEETING CLOSES

15:00 – 15:30 Tea/coffee break

15:30 – 17:00 Satellite seminar on human monkeypox
– Pierre Formenty

Annex 2. List of participants

Advisory Committee

Dr Isao Arita, Chairman, Agency for Cooperation in International Health, Kumamoto City, Kumamoto, Japan

Mr Robert Drillien, Directeur de Recherche à l'INSERM, IGBMC - Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, Cedex, France

Professor Mariano Esteban, Head of Poxvirus and Vaccines, Centro Nacional de Biotecnologia, Campus Universidad Autonoma, Cantoblanco, Madrid, Spain

Dr David H. Evans, Professor and Chair, Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada

Dr James W. LeDuc, Director, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, USA

Dr Akhilesh Chandra Mishra, Director, National Institute of Virology, Pune, India

Professor Georg Pauli, Head of the Center of Biological Safety, Zentrum für Biologische Sicherheit (ZBS) Hochpathogene virale Erreger (ZBS 1) Robert Koch Institut, Berlin, Germany

Dr André D. Plantinga, Senior Project Manager, Vaccine Development, Netherlands Vaccine Institute (NVI), Bilthoven, The Netherlands

Dr Tony Robinson, Senior Principle Research Scientist**,** CSIRO Sustainable Ecosystems, Canberra, Australia

Dr Li Ruan, Chairman of Department of Viral Genetics and Immunology, Chinese Center for Disease Control and Prevention, China

Professor Lev S. Sandakhchiev, Adviser to General Directors, State Research Center of Virology and Biotechnology, VECTOR, Koltsovo, Novosibirsk region, Russian Federation

Dr Hermann Schatzmayr, Head Virology Department, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

Dr Robert Snoeck, Senior Researcher, Katholieke Universiteit Leuven, Rega Institute for Medical Resarch, Leuven, Belgium

Professor Geoffrey L. Smith, Professor of Virology**,** Wellcome Trust Principal Research Fellow, Department of Virology, Faculty of Medicine, Imperial College London, London, England

Professor Robert Swanepoel, Consultant Virology, National Institute for Virology, Sandringham, South Africa

Professor Muyembe Tamfum,[1]Director, Institut National de Recherche Bio-Médicale (INRB), Kinshasa, Democratic Republic of the Congo

[1] unable to attend

Dr. Oyewale Tomori, Redeemer's Univeristy, Lagos, Nigeria

Professor Dr Prasert Thongcharoen, Professor in Virology**,** Division of Virology, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

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

Advisors to the Committee

Dr Antonio Alcami, Centro Nacional de Biotecnología (CSIC), Campus Universidad Autonoma, Madrid, Spain

Dr Annette Arnberg, Bavarian Nordic A/S, Kvistgard, Denmark

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Dr Kalyan Banerjee, Vice President, Maharashtra Association of Cultivation of Science, Agharkar Research Institute, Pune, India

Dr Philip Bedford, Senior Vice President, Clinical Operations and Regulatory Affairs, Acambis Research Limited, Cambridge, England

Dr Peter D. E. Biggins, Head of IRS, DERA-CBD, CB Systems, Porton Down, Salisbury, United Kingdom

Dr Mike Bray,[1] MD MPH, Medical Officer, Biodefense Clinical Research Branch, NIAID, National Institutes of Health, Bethesda, USA

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Dr Karoline Dorsch, Executive Secretary, Swiss Expert Committee for Biosafety (EFBS/CFSB) Bern, Switzerland

Dr Joseph J. Esposito, Coordinator of Collaborative Research, Biotechnology Core Facility Branch, Centers for Disease Control and Prevention, Atlanta, USA

Professor Daniel Garin, Head of Virology Lab, CRSSA Emile Pardé, Grenoble, France

Dr M. Garstang, Director of Marketing, Acambis, Peterhouse Technology Park, Cambridge, England

Dr Lance Gordon, President & CEO, VaxGen, San Francisco, USA

Dr Donald A. Henderson,[1] Director Johns Hopkins Center for Civilian Biodefense Studies, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, USA

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Dr John W. Huggins, Chief, Department of Viral Therapeutics, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland, USA

Dr Dennis E. Hruby, Chief Scientific Officer, SIGA Technologies Inc.,Corvallis, Oregon, USA

Dr Lauren Iacono-Connors,[1] Senior Advisor to the Center Director, CBER, Food and Drug Administration, Rockville, Maryland, USA

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Dr Nina Tikunova, Head of Laboratory, State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk region, Russian Federation

Dr David Ulaeto, Scientific Leader, DERA-CBD, Biomedical Sciences, Porton Down, Wilshire, England

Observers

Dr. Lisa E. Hensley, Microbiologist, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland, USA

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Dr D. Lavanchy, EPR/EDP
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Dr M. Chu, EPR/EDP
Dr N. Previsani, EPR/LYON
Dr R. Wittek, EPR/EDP
Dr P. Greenaway, Independent Consultant, United Kingdom