



WHO Advisory Committee on Variola Virus Research

Report of the Fifteenth Meeting

Geneva, Switzerland

24–25 September 2013



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WHO Advisory Committee on Variola Virus Research
Report of the Fifteenth Meeting, Geneva, Switzerland, 24–25 September 2013

Executive Summary

On 24 and 25 September 2013 the Advisory Committee on Variola Virus Research held its fifteenth meeting. In addition to its regular annual review of the virus stocks held in the two authorized repositories, the research undertaken and the proposals submitted, the Advisory Committee took the opportunity to review all research conducted over the past three years. The intention behind this in-depth review was to contribute to preparations for the forthcoming discussion at the Sixty-seventh World Health Assembly in May 2014 on the timing of the destruction of variola virus stocks.

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

The Advisory Committee was also provided with updates on the use of live variola virus for the development of: diagnostic tests, one animal model, smallpox vaccines, and antiviral agents and therapeutics. Representatives of two pharmaceutical companies described candidate antiviral agents (tecovirimat and brincidofovir) that were in an advanced stage of development. Information presented included data on efficacy, safety, stability and large-scale manufacturing capacity. Work is continuing to provide the data that are needed to satisfy the requirements for regulatory approval. A representative of another pharmaceutical company provided an update on its vaccine that had been licensed in the 28 Member States of the European Union, plus Iceland, Liechtenstein and Norway, in August 2013, with the indication for active immunization against smallpox of all adults.

Members of the Committee were asked to consider whether live variola virus was needed for further essential research for public health benefit on diagnostics for smallpox. The majority view within the committee was that there was no need to retain live variola virus for development of further diagnostics for smallpox.

Members of the Committee were asked to consider whether live variola virus was needed for further essential research for public health benefit on vaccines against smallpox. The majority view within the committee was that there was no need to retain live variola virus for the development of safer smallpox vaccines beyond those studies already approved.

Members of the Committee were asked to consider whether live variola virus was needed for further essential research for public health benefit on antivirals for smallpox. The majority view within the committee was that live variola virus was needed for the further development of antiviral agents against smallpox.

The CDC's "use to completion" of 70 of its 420 variola virus stocks in the process of approved research has set a potential precedent for the progressive reduction of all live virus material being held in the two repositories as a means of meeting the request of the World Health Assembly.

The Advisory Committee was appraised of other consultations, the outcome of which would have relevance to discussions to be held at the Sixty-seventh WHA. These consultations were as follows:

- The Advisory Group of Independent Experts to review the smallpox research programme (AGIES) would be reconvened in November 2013 to consider the recommendations and decisions of the Advisory Committee.
- An expert consultation aimed at reviewing the evidence on smallpox vaccines and proposing recommendations for the size and composition of the WHO smallpox vaccine stockpile had been convened on 19 and 20 September 2013. That consultation will present its conclusions and recommendations to the Strategic Advisory Group of Experts (SAGE) on Immunization for consideration at meeting in November 2013.

In further preparation for the discussions at the World Health Assembly in May 2014, the reports of 15th meeting of ACCVR, AGIES and SAGE, as well as that of the Secretariat, would be submitted to the Executive Board of WHO for consideration at its 134th session in January 2014.

1. Report of the Secretariat

- 1.1. The WHO Advisory Committee on Variola Virus Research met on 24 and 25 September 2013 under the chairmanship of Professor G.L. Smith, with Mr D. FitzSimons as Rapporteur.
- 1.2. Dr K. Fukuda opened the meeting and advised that this fifteenth meeting of the Committee would combine the usual annual review of the virus stocks held in the repositories, the research undertaken and proposals submitted with a consideration of the research conducted over the past three years, in view of the forthcoming discussion at the Sixty-seventh World Health Assembly in May 2014 regarding the timing of the destruction of variola virus stocks. In addition, the Advisory Group of Independent Experts to review the smallpox research programme (AGIES) would be reconvened in November 2013 to consider the recommendations and decisions of the Committee. The reports of both bodies as well as that of the Secretariat would be submitted to the Executive Board at its 134th session in January 2014. Furthermore, an expert consultation aimed at reviewing the evidence on smallpox vaccines and proposing recommendations for the Strategic Advisory Group of Experts (SAGE) on Immunization had been convened on 19 and 20 September 2013; the expert consultation consider the size and composition of the WHO smallpox vaccine stockpile.
- 1.3. Dr A. Costa reported on the work of the Secretariat. The reports of the biosafety inspections in 2012 of the two repositories of variola virus, in the Russian Federation and the United States of America, had been finalized and would be posted soon on the WHO web site. Terms of reference had been drafted for the orthopoxvirus laboratory network and consultations were being held with WHO regional offices on candidate laboratories to be part of this network. A protocol for the means and date of destruction of non-infectious cloned fragments of variola virus DNA that were being held in South Africa has been prepared.

2. Update on WHO-approved research proposals

- 2.1. Dr R. Drillien summarized the 10 proposals submitted to the Advisory Committee's scientific subcommittee in 2012-2013 and recommended for approval (see Annex 11). Most concerned work with live variola virus but a couple related to matters relating to distribution of variola viral genes.

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

- 3.1. Professor A. Sergeev reported on the status of the collection of strains and materials held in the variola virus repository in the SRC VB VECTOR, Koltsovo, Novosibirsk Region, Russian Federation. The collection continued to contain 120 strains and 696 registered samples. Standard operating procedures and emergency plans had been elaborated. Research in 2013 had included use of the Ind-3a strain of variola virus for

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

assessing the antiviral properties of various synthetic compounds, the neutralizing activity of sera from vaccinated subjects, and susceptibility of mice to viral infection. Such research was planned to continue into 2014.

- 3.2. Dr I. Damon reported on the status of the collection at the US Centers for Disease Control and Prevention (Atlanta, Georgia). No new seed pool had been added between 2012 and 2013, but working stocks of six purified variola virus stocks had been prepared and stored. Live virus had been used in approved research projects on less reactogenic, third-generation vaccines, development of a mouse model of smallpox, the identification of potential host targets for therapeutic intervention, evaluation of antiviral agents, and preparation of materials for diagnostic tools. This research included 135 removals of material from the collection for genomic sequencing without propagation, 70 of which were used to completion and so represent a reduction in the total number of virus samples stored in the facility, although non-infectious DNA fragments from these samples remain in some cases.² Archived material had been or could be used for genomic sequencing. Investigation of viral material in scabs provided no unequivocal evidence that such materials contained subpopulations of variola virus. It was also noted that one isolate classified as a variola virus strain was shown by DNA sequencing to be a vaccinia virus isolate.

4. Update on laboratory diagnostic tests

- 4.1. Dr Damon presented an update on the use of live variola virus for the development of diagnostic tests for variola virus based on nucleic acid or protein detection systems. Although a variola virus-specific PCR-based assay set, quality managed through the US laboratory response network, had been demonstrated to be sensitive and specific in 2010, subsequent sequencing of Eurasian cowpox virus strains rendered one assay signature no longer variola virus specific and so additional refinement was being undertaken. Validation of two specific variola virus real-time PCR assays has been completed on two platforms that have undergone regulatory review with two different master mixes. Using a variola virus-specific monoclonal antibody (E2), a variola virus-specific antigen capture assay has been developed and its sensitivity is being refined. Using two live virus preparations, sensitive detection to 100,000 particles was identified using a standard secondary conjugate; gamma-irradiated virus preparations of the same viruses gave more variable results. Additional variola-specific reagents are being evaluated, as are more sensitive detection methods. Hybridomas from the 2000 immunization protocol were regrown and resubcloned. To date, four monoclonal antibodies were shown to specifically recognize variola (n=1), or recognize variola more avidly than vaccinia (n=1) or monkeypox (n=2). Cowpox and camelpox virus isolates were not recognized by any of these monoclonal antibodies in ELISA or western analyses. In some cases, the viral target has been identified. With Lawrence Livermore National Laboratory scientists, a bioinformatics approach to identify possible variola specific monoclonal antibody targets amongst surface or envelope proteins yielded at least 36 potential targets. These targets will be exploited for monoclonal antibody development using an outside partner organization with expertise in this approach to monoclonal antibody development. Finally, she outlined

² See Annex 2 for a full list of the viral isolates and stocks concerned.

work on the monkeypox specific serological detection in ELISAs with expressed peptides as antigens. Results so far provided proof of concept for the approach, which could be adapted for smallpox.

5. Update on animal models

- 5.1. Dr Damon reported progress on evaluation of the CAST/EiJ mouse model for variola virus infection. These mice have a deficient gamma-interferon response. After intranasal infection with 5×10^4 pfu, 5×10^5 pfu or 5×10^6 pfu these mice shed viable variola virus and showed signs of disease, including loss of up to 15% body weight before recovering. However, the weight loss was variable and was affected by the age of mice; mice 9 weeks of age showed fewer signs and symptoms than animals 8 weeks of age. All animals challenged with live virus had a high anti-variola virus antibody titre at 21 days, the end of the study. Further planned work will look at infection in younger mice.

6. Update on vaccines

- 6.1. Dr L. Bulychev, using the Ind-3a variola virus strain, had investigated neutralizing antibody titres in the sera of three groups of subjects vaccinated with a new orally administered recombinant vaccine, Revax-BT, or with currently licensed vaccines (a killed vaccine and live vaccine). The first group of subjects consisted of individuals not previously vaccinated against smallpox who received vaccination with Revax-BT. The second group consisted of individuals not previously vaccinated who received a first vaccination with the killed licensed vaccine, followed by booster vaccination with the live licensed vaccine. The third group was composed of individuals previously vaccinated against smallpox; during this study they received their revaccination with the licensed live vaccine. The antibody titre in the serum of a specific human subject (vaccinee) measured in the neutralization test with variola virus did not significantly differ from the antibody titre in the same serum measured in the neutralization test with vaccinia virus. There was a significant increase in the geometric mean titre of anti-smallpox antibodies in the sera of individuals of Group 3 compared with Groups 1 and 2.
- 6.2. Dr V. Olson reported results of collaborative studies with the National Institute of Infectious Diseases in Japan and pharmaceutical industry scientists on the continued evaluation of vaccination regimens for third-generation smallpox vaccines, specifically LC16m8, for neutralization. The neutralizing capacity of a limited number of vaccinees' sera was compared between different strains of variola (from both primary clades) and different vaccines (Dryvax (n=4) versus LC16m8 (n=10)). LC16m8 vaccination induces neutralizing immune response to live variola virus; although there were differences in variola 50% neutralization geometric mean titres (GMTs) between Dryvax and LC16m8 vaccinee sera, these did not reach statistical significance. There were significant differences in the LC16m8 vaccinee sera variola GMTs if primary clade 1 or primary clade 2 viruses were used in neutralization. Dr Olson reported on the high-content orthopoxvirus neutralization assay (HCOVNA) that was optimized and shown to be reproducible and robust. It can be used for

multiple orthopoxviruses, needs smaller volumes of sera and has a large dynamic range.

- 6.3. Dr L. Wegner reported that Imvanex® (Imvamune® in the USA) had been licensed in the 28 Member States of the European Union, Iceland, Liechtenstein and Norway in August 2013, with the indication for active immunization against smallpox of all adults. A submission for approval had been filed in May 2011 in Canada, and approval is expected by the end of 2013. In the USA a pre-Emergency Use Authorization is in place that would allow use of the vaccine in certain immunocompromised populations (i.e. HIV and atopic dermatitis regardless of age and including pregnant women and children). It has been included in the US Strategic National Stockpile, – an initial order of 20 million doses was made and, in April 2013, the US government ordered another 8 million doses to replace expiring product. The vaccine has been administered to 7000 people with no safety concerns. The approval offers a unique opportunity to protect citizens who are not candidates to receive replicating vaccines.
- 6.4. Dr H. Yokote described recent research on the third-generation attenuated, replication-competent vaccinia virus vaccine LC16m8, licensed in Japan since 1975 and currently stockpiled in that country. It has been administered to some 90,000 infants in the past and recently to more than 8000 military personnel in Japan and 125 adults in USA without severe adverse effects. Given to people who had previously been immunized against smallpox (even several decades ago), LC16m8 induced an effective booster response irrespective of how many doses they had previously received. In mice vaccinated first with LC16m8 and revaccinated with another LC16m8 vaccine, the titres of neutralizing antibodies in the animals were comparable to those in animals vaccinated with other vaccinia vaccines. In animals vaccinated with LC16m8 at primary vaccination and revaccinated with a conventional vaccine (e.g. Lister), skin lesions were still observed in a diminished size but the immune response was comparable to that in animals vaccinated twice with a conventional smallpox vaccine. In other words, LC16m8 is an adequate vaccine to be stockpiled for preparedness against possible smallpox attacks and is expected to work effectively when used singly or in combination use with first- and second-generation vaccines. The manufacturing capacity in Japan is 80 million doses a year.

7. Update on antiviral agents and therapeutics

- 7.1. Dr L. Bulychev outlined the testing of more than 100 synthetic compounds for antiviral activity against surrogate orthopoxviruses for variola virus. Thirty promising compounds had been further tested in vitro against the Ind-3a variola virus strain, with one, NIOCH-268 (having some similarity to ST-246), showing high activity.
- 7.2. Dr V. Olson reported on the use of live variola virus to evaluate antivirals against smallpox. Multiple compounds that act at different stages of the viral life cycle have been investigated previously: the tyrosine kinase inhibitor imatinib/Gleevec (which is FDA licensed for the treatment of chronic myelogenous leukemia and other malignant neoplasms) blocks egress of extracellular enveloped variola virus; tecovirimat (also called ST-246, Arestvyr) blocks wrapping of intracellular mature virus; CMX001 (an

acylated form of cidofovir) blocks variola virus DNA replication; and crude *Sarracenia purpurea* extract inhibits early variola viral transcription. Dr Olson updated progress on the evaluation against variola virus for: CMX001 inhibition; the proteasome inhibitor bortezomib (Velcade), which blocks formation of vaccinia virus replication factories; and pyridopyrimidinones, a new class of compounds that inhibit intermediate and late viral gene expression of several orthopoxviruses. The main findings included the observation that the IC₅₀ of CMX001 was 100 times lower than that for cidofovir against variola virus, the therapeutic index of proteasome inhibitors was lower than expected against variola virus, and the pyridopyrimidinones blocked variola virus multiplication post viral DNA replication with an IC₅₀ lower than the concentration that induced cellular toxicity. It was noted that the development of both CMX001 and tecovirimat as smallpox countermeasures were very far advanced compared with other candidate compounds.

- 7.3. Dr J. Kindrachuk described the results of kinome analyses, with particular focus on the differential modulation of host cell signalling responses following variola virus or monkeypox virus infection. Kinases are a major target for the design and development of new therapeutics (the FDA has approved 25 kinase inhibitors and more than 400 are in clinical development), and the US National Institutes of Health supports repurposing drugs approved by the FDA. Dr Kindrachuk's team undertook functional network analysis of variola virus kinase data through bioinformatics analysis and biological validation with traditional western blot approaches and kinase inhibitor assays. Studies using variola virus and monkeypoxvirus in human monocytes showed that the two viruses differentially modulate host responses to viral infection, raising the question of whether infection with monkeypoxvirus was a true surrogate for smallpox. For the future it is planned to examine the therapeutic potential of FDA-licensed kinase inhibitors against variola virus and monkeypox virus to further clarify targets that are conserved or differentially employed by these viruses. Further work will also expand kinome analysis into multiple host cell targets of the virus.
- 7.4. Dr D. Hruby described progress of tecovirimat (ST-246) towards regulatory approval, its inclusion in the US Strategic National Stockpile, and results from a study where tecovirimat was concurrently administered with ACAM2000 vaccine in non-human primates. The antiviral had shown to have activity against six orthopoxviruses in more than 40 different animal challenges. The oral drug appears to be safe and well tolerated, and has investigational new drug status. Experiments in monkeys infected with monkeypoxvirus showed that the antiviral agent results in 100% survival whereas administration of the variola virus vaccine ACAM2000 did not. Simultaneous administration of vaccine and antiviral did not affect the immune response to the vaccine and protection was also 100%. The manufacturing company was awarded a contract worth up to US\$ 2.8 billion in 2011 to supply the US Strategic National Stockpile; so far 725,000 of the initial 2 million courses ordered have been delivered. Protocols are in place for the compassionate use of tecovirimat in case of adverse event following vaccination with smallpox vaccines. The company has worked with the US regulatory authorities and elaborated a feasible approach to approval under the Animal Rule, which included efficacy studies in an intradermal rabbitpox model and non-human primate model.

- 7.5. Dr L. Trost outlined the development of brincidofovir (CMX001) for smallpox and other indications. It is active against the five families of double-stranded DNA viruses, including all orthopoxviruses, has a high barrier to development of resistance, is stable for several years, and has been administered to more than 800 people. A comprehensive nonclinical safety assessment has been completed. Since 2009 it has been used under Emergency IND regulations and in an open-label study with 215 patients to treat infections caused by cytomegalovirus, adenovirus, BK virus, JC virus, multiple other herpes viruses and a case of progressive vaccinia in a military vaccinee. Following discussions with the US FDA, the development of brincidofovir for an indication of treatment of smallpox will use the intradermal rabbitpox model and the intranasal ectromelia model in mice. Brincidofovir was effective in both models when treatment was initiated 3-5 days after infection, and survival correlated with dose. On the basis of the results in animal models it is predicted the concentrations of CMX001 and the active metabolite cidofovir-diphosphate needed for treatment of smallpox can be achieved in humans with doses currently being evaluated clinically for treatment of other diseases. Tablet and liquid formulations are expected to be commercially available. Manufacturing on a commercial scale has been validated.

8. Regulatory issues

- 8.1. Dr L. Borio reported the FDA's regulatory progress in facilitating the development and approval of smallpox medical countermeasures since the Committee's previous meeting. She provided a brief status report on all smallpox medical countermeasures with FDA's purview, in the areas of diagnostics (nucleic acid based orthopoxvirus assays were approved), vaccines (ACAM2000 was licensed; while WetVax, liquid frozen and freeze-dried modified Vaccinia Ankara (MVA) and LC16m8 were under Investigational status), and treatments (vaccinia immunoglobulin was licensed; while tecovirimat and brincidofovir were under Investigational status). The focus of the FDA's interactions with developers of these countermeasures in the past year had been on providing feedback on proposed studies to support safety, pharmacokinetics and animal model efficacy studies for antiviral agents and efficacy studies for the attenuated vaccine. She noted that regulatory decisions for smallpox medical countermeasures are inherently associated with scientific uncertainties, but good progress had been made.
- 8.2. Dr R. Ruepp outlined the European Medicines Agency's activities on smallpox. Its pre-authorization tools include scientific advice, orphan drug designation, and agreement of a paediatric investigation plan. However, as the current incidence of smallpox within the EU is 0, orphan drug designation cannot be given for therapeutics or vaccines against smallpox. Orphan designation was given to Tecovirimat in May 2012 for the treatment of cowpox infection. For Imvanex (a live MVA vaccine) a paediatric investigation plan is in place, which targets the following indication: "active immunisation against smallpox infection and disease in the paediatric population including HIV patients and patients with atopic dermatitis". The requirement to gather certain data in children was deferred until the time of a potential outbreak of smallpox. The European Commission granted a decision for an EU Marketing Authorization under exceptional circumstances for Imvanex in July 2013 for the active immunization of adults against smallpox. This followed a positive

opinion from the EMA's Committee for Medicinal Products for Human Use (CHMP), which concluded mainly from the assessment of five clinical studies that the benefits of Imvanex outweighed its risks. The Marketing Authorization was accompanied by specific obligations, which include the completion of: an observational, non-interventional post-authorization safety study for the prophylactic vaccination with Imvanex in adults, and an observational, non-interventional post-authorization safety and efficacy study for the prophylactic vaccination with Imvanex following the re-emergence of circulating smallpox infections. In addition, clinical data on lot consistency and comparison with ACAM2000 are required to be submitted post authorisation.

9. Smallpox diagnostic network

- 9.1 Dr J.-C. Piffaretti described progress in establishing a global smallpox laboratory network for early smallpox diagnostics. Terms of reference had been agreed and criteria for inclusion of a candidate laboratory have been defined. Selection of the candidate laboratories is in progress and the procedures to be followed are being elaborated. An ad hoc Independent Technical Group has been formed to agree on a standard diagnostic method for molecular diagnostics of orthopoxviruses and molecular identification of variola virus. The Group has agreed a set of principles for the methods, including use of real-time PCR, validation of the assay with the most common orthopoxviruses that infect humans, use for clinical specimens only, the target of producing a kit format, and a two-step assay scheme (first, for identification of the most frequent orthopoxviruses that infected humans, but including variola virus, and, secondly, specific identification of variola virus). The proposal for the molecular assay will be finalized during the current biennium 2013-2014.

10. WHO smallpox vaccines stocks

- 10.1. Dr A. Costa reviewed the development of the WHO smallpox vaccine stockpile and gave an update on its status. At present a total of 32 million doses had been pledged. The pledges included the vaccines used during the eradication campaign, ACAM2000, LC16m8 and Imvamune. The WHO physical stockpile of 2.7 million doses (2.4 million doses of vaccines used during the eradication campaign and 300,000 doses of ACAM2000) is held in Switzerland. The WHO stockpile included 12 million bifurcated needles. Other countries have their own stockpiles and there may be stockpiles of between 570 and 720 million doses worldwide. The production capacity of the vaccine manufacturers amounts to about 255 million doses a year although some production lines would have to be restarted. At the recent expert consultation aimed at reviewing the evidence and proposing recommendation for SAGE's review a series of questions was posed about which vaccine to recommend in case of an outbreak and which groups should it be provided to, which vaccine should be used for prevention (for groups such as first aiders and military personnel), and what should be the immunization schedule. The report of that consultation would be finalized in time for the SAGE meeting in November 2013.

11. Update on variola virus repositories biosafety inspections

- 11.1. Dr N. Previsani described the preparations for the planned biosafety inspections of the two repositories of variola virus (CDC, Atlanta, Georgia, USA, and VECTOR, Koltsovo, Novosibirsk Region, Russian Federation) in 2014. The reports of the previous inspections in 2012 would soon be published on the WHO web site. The Secretariat was finalizing arrangements for the destruction of cloned variola virus DNA fragments that were stored in South Africa, including risk assessment, date (provisionally agreed for December 2013), composition of the team to witness the event, and the updating of the certification procedure set out in the Report of the Meeting of the Ad Hoc Committee on Orthopoxvirus Infections in 1994. She added that WHO's experience in the eradication of smallpox and work towards eradication of poliomyelitis had been useful to FAO and OIE following the eradication of rinderpest.

12. General discussion

- 12.1. The Chairman summarised the current position regarding the development of diagnostics, anti-viral drugs and vaccines and then invited comments from all participants in the meeting on the need to retain live variola virus for additional essential research for public health benefit.

Diagnostics

- 12.2. Summary. Although a variola virus-specific PCR-based assay set, quality managed through the U.S. laboratory response network, had been demonstrated sensitive and specific in 2010, subsequent sequencing of Eurasian cowpox virus strains rendered one assay signature no longer variola virus specific and so additional refinement was being undertaken. A multiplex PCR-based variola/orthopoxviruses assay set was developed and licensed in Russia in 2011. Systematic progress on new assay/kit validation, including discussions with regulatory authorities, has been made towards this goal. Work continues with the development of protein-based detection systems, but currently these lag behind the nucleic-acid-based systems in terms of sensitivity.

During the subsequent discussion the following questions were raised:

How representative were the known sequences of variola virus and were additional sequences needed? It was stated that the ~50 sequences available hitherto were very highly conserved and as the available tests detected, or were predicted in silico to detect, all these, it was considered unlikely that any other unsequenced variola viruses would be sufficiently different to avoid detection by existing tests.

Was a test that was absolutely specific for variola virus, rather than one that detected variola viruses and a few strains of cowpox virus, necessary? Some felt that a truly specific kit was desirable but not essential. Any first test would be followed up by sequencing to confirm or refute the identification of variola virus.

Were protein-based assays under development likely to have sufficient specificity and sensitivity? It was noted that some protein-based assays used for diagnosis of other pathogens suffer from a higher rate of false-positive reactions and have lower sensitivity and specificity. The use of a rapid protein-based assay as primary diagnostic test might help facilitate the movement of specimens to confirmatory laboratories for final assessment.

Arguments for the retention of live virus identified the need for its use in quality assurance and validation of new assays, specifically virus particle/ antigen-capture tests, and the use of genomic variola virus DNA in the assessment of different nucleic acid assays.

Arguments against its retention included the existence of well-characterized assays, the lack of need to produce new variola virus-specific and better performing assays, and the fact that DNA fragments can be synthesized.

Members of the Committee were asked to consider whether live variola virus was needed for further essential research for public health benefit on diagnostics for smallpox. The majority view within the committee was that there was no need to retain live variola virus for development of further diagnostics for smallpox.

Smallpox vaccine

- 12.3. Summary. Progress has been substantial on the development of safer smallpox vaccines. Two smallpox vaccines have been licensed (ACAM2000 in USA in 2007 and LC16m8 in Japan in 1975) for use in healthy individuals. In addition, during the last year an MVA-based vaccine has received marketing authorization under exceptional circumstances from the European Commission for active immunization against smallpox infection and disease in persons 18 years of age and older. Immunocompromised subjects are now eligible for vaccination.

Arguments against retaining live variola virus included the existence of good vaccines that have been licensed.

An argument for retention of live variola virus was for the evaluation of vaccine efficacy in new animal models for smallpox that might be developed or for neutralization assays. It was pointed out that variola neutralization studies did not form the basis of approval of the MVA vaccine for the regulatory review process; however, these studies had been requested during regulatory review as scientifically important. The FDA pointed out that the licensure of MVA in the US will be based on demonstration of non-inferiority to the licensed ACAM2000 vaccine. The FDA has requested additional supportive studies, including variola neutralization assays. This work would not begin until 2014, after clinical study enrolment was completed.

Members of the Committee were asked to consider whether live variola virus was needed for further essential research for public health benefit on vaccines against smallpox. The majority view within the committee was that there was no need to retain live variola virus for the development of safer smallpox vaccines beyond those studies already approved.

Antiviral agents

- 12.4. Major advances have been made in this field. Two compounds, tecovirimat (ST-246; Arestvyr) and brincidofovir (CMX001), which are active and have different mechanisms of action, are well advanced along the regulatory pathway to licensure for smallpox. Tecovirimat has been tested in many animal models and has demonstrated unequivocal protection against orthopoxviruses, including variola virus, in these models. The US FDA has requested additional limited studies with the intradermal rabbitpox model and the intranasal ectromelia model in mice but stated that, at present, more work with live variola virus is not needed for these compounds. Despite not being licensed, ST-246 has been purchased for the US Strategic National Stockpile. A series of other potential compounds are in much earlier stages of development as medical countermeasures against smallpox.

Questions discussed included:

Is work on additional compounds essential in view of the very advanced stages of development for ST-246 and CMX001? Some members of the committee felt that until licensure is reached for the lead compounds, live virus should be retained in the event that the lead compounds fail to be licensed and identification/development of other compounds is required. Others felt that it was so unlikely that the lead compounds would not be licensed that live virus was no longer needed.

Was development of additional animal models for smallpox essential? Some members argued that this aim was not a justification for retaining live variola virus. In many cases, it may be feasible to use other orthopoxviruses models as surrogates. Others felt that the development of a better animal model was highly desirable.

Members of the Committee were asked to consider whether live variola virus was needed for further essential research for public health benefit on antivirals for smallpox. The majority view within the committee was that live variola virus was needed for the further development of antiviral agents against smallpox.

Partial destruction of the variola virus stocks

- 12.5. The views on retention of live variola virus raised the question of how much of the stored viral stocks needed to be retained – the complete repertoire or a representative selection of isolates? The collaborating centres agreed to discuss this. It was noted that reduction of the number of isolates in one of the repositories had already happened during the last year, with the “use to completion” by the CDC of 70 of its 420 variola virus stocks in the process of approved sequencing work, which preserved the essential genetic information about the destroyed isolates. The chairman asked representatives from the two collaborating centres to consider retaining only an appropriate representative subset of the existing live variola viruses and to destroying other strains, perhaps after extraction of nucleic acid for possible future genomic analysis.

Concluding remarks

- 12.6. The approved smallpox research programme has made much progress since 1999 and is approaching its end. The Committee underlined the extraordinary scientific progress that had been made in the past decade and applauded the work of the scientists involved. Members discussed the difficulty in distinguishing between matters that were scientifically and medically interesting rather than essential for public health need.

The members of the Committee were, by and large, in agreement that live variola virus need no longer be retained for further essential research for public health benefits on diagnostics and vaccines, but saw the necessity to retain live variola viruses for further work on antivirals. The destruction by the CDC of 70 of its 420 variola virus stocks in the process of approved research has set a potential precedent for the progressive reduction of all live virus material being held in the two repositories as a means of meeting the request of the World Health Assembly while safeguarding the protection of populations through further development of antiviral agents against smallpox.

Annex 1: Summary of presentations

Update on research proposals submitted to WHO in 2012/2013 and recommended for approval

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

5 December 2012 (review date)

- **Project from Fox Chase Cancer Center, Philadelphia, United States of America**
Request for the cloned gene for the variola virus (VARV Type I interferon binding protein (T1-IN bp))
- **Project from VECTOR, Novosibirsk, Russian Federation**
Assessment of the neutralizing activity of vaccine blood sera using live variola virus

11 February 2013 (review date)

- **Projects from CDC, Atlanta, United States of America**
Use of variola virus to determine whether CAST/Eij mice are a suitable animal model for human smallpox

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

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

Use of variola virus to evaluate antivirals against variola

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

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

25 March 2013 (review date)

- **Project from VECTOR, Novosibirsk, Russian Federation**
Discovery of new antivirals for smallpox treatment and prevention

30 July 2013 (review date)

- **Project from CDC, Atlanta, United States of America**
Validation of viral nucleic acid removal from in vitro RTS protein reactions with Benzonase treatment

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

Prof. Alexander N. Sergeev

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

Organization of and experimentation with the Russian variola virus (VARV) collection at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at SRC VB VECTOR is in compliance with national and international requirements and the recommendations of the WHO Global Commission. Instructions regulating research, as well as all maintenance and control procedures, have been developed on the basis of the documents listed above. Plans have been developed for anti-epidemic measures and response to accidents. Emergency teams have been established for activation in case of accidents and emergency situations.

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

According to an inventory inspection, the Russian collection of variola virus strains contains:

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

The total number of registered stored units is 696.

In 2013 the Ind-3a variola virus strain was grown in Vero cells and it was then used to assess antiviral properties of chemically synthesized compounds, in cell culture; the neutralizing activity of sera collected from vaccinated individuals, in cell culture; and susceptibility of mice to VARV.

Research using live variola virus is planned in 2013 - 2014 to:

- discover new antiviral chemically synthesized compounds for treatment and prevention of smallpox;
- assess variola virus neutralizing activity of sera from those vaccinated against smallpox;
- develop animal models to study the efficacy of therapeutic and preventive products against smallpox.

Report on the variola collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention Atlanta, Georgia, United States of America

Victoria Olson, Paul Hudson, Ashley Kondas, Zachary Reed, Zachary Braden, Scott Smith, Christine Hughes, Hui Xhao, Jason Gao, Kimberly Wilkins, Yu Li, Inger Damon

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

The World Health Organization (WHO) Collaborating Center for Poxviruses in Atlanta, GA continues to maintain one of two consolidated, international collections of variola virus strains. The majority of these viruses were clinical samples 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 that has remained largely untouched. Secure databases, which address WHO recommendations as well as U S Select Agent requirements, have been constructed to track usage of variola virus. Annual reports on the status of these collections are provided to WHO. No new variola virus seed pools were added to the inventory between 2012 and 2013. Working stocks of six purified variola virus strains (IND64_vel4, BSH74_sol, SLN68_258, SOM77_ali, BRZ66_39, and JAP51_hpr) have been prepared and stored. One aliquot of each underwent inactivation by gamma irradiation for use in monoclonal antibody screening for protein-based diagnostics. WHO-approved research activities that have used variola virus or products from earlier studies using variola virus from the inventory within the last year have focused on finalization of sample analysis of previously performed animal studies, tissue culture analysis of promising compounds for anti-variola virus activity, evaluation of the variola virus elicited host kinome response to look for potential therapeutic targets, optimization of protein-based diagnostic assays, and evaluation of sera from vaccination regimens to evaluate efficacy based on variola virus neutralization.

Variola virus has been used to support research on the following approved protocols:

1. Use of live variola virus to support less-reactogenic vaccine development, continued evaluation of third-generation vaccines
 - 18 removals from 4 strains (Solaiman, Harper, V69-1, V68-258)
2. Use of live variola virus CASTeIJ
 - 30 vials of necropsy tissues removed for processing (3/7/13)
3. Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention
 - 1 removal from 1 strain (Harper)
4. Use of live variola virus to evaluate antiviral agents

- 26 removals from 6 strains (Solaiman, V66-39, SOM, v68-258, Stillwell, Butler)
5. Use of live variola virus to maintain and regenerate non-infectious variola-derived materials for diagnostic development support
- DNA diagnostics
 - 135 removals of 135 strains (see Table 1)
 - Protein diagnostics
 - 21 removals of 6 strains (Solaiman, Harper, India 7124, v66-39, SOM, v68-258)

Table 1. List of variola virus material used for genomic sequencing without propagation: 135 removals of 135 stains, 70 of which were used to completion (text in bold, blue cells).

| | | | | | | | | | | | |
|--------------|--------------|--------------|------------------|------------------|--------------|---------------|----------|-----------|-----------|------------|---------------------|
| 080 | 285 | BEL | Calc | Iran 9883 | LEF | Ramjan | V67-96 | V69-341 | V72-I-38 | V74-I-108 | WIY |
| 086 | 32/62 | Bombay | CAM | Islambibi | LEV | ROB | V67-I-20 | V69-6 | V72-I-394 | V74-I-14 | Yamamoto E36 |
| 1/61 | 340 | Br 10 | Djib | JON | LUK | Rub | V67-I-51 | V69-I-418 | V72-I-412 | V74-I-142 | YOU |
| 12/61 | 389 | Br13 | DUN | JOS | MAM | Sajeda | V68-104 | V70-I-245 | V72-I-71 | V74-I-205 | |
| 126 | 5008 | Br15 | Eth 11 | K 5/67 | Misba | Sharma | V68-197 | V70-I-112 | V72-I-74 | V74-I-65 | |
| 141 | 6/63 | Br18 | Eth 23 | K1628 | MUZ | Subra | V68-207 | V70-I-60 | V72-I-80 | V74-I-91 | |
| 17/61 | 8/61 | Br23 | Eth 9 | Kali Muthu | New Delhi | SUG | V68-231 | V71-I-213 | V73-I-08 | V75-I-109 | |
| 180 | Abid | Br3 | Eth24 | KAS | NJO | TIN | v68-254 | V72-I-005 | V73-I-140 | V75-I-148 | |
| 186 | Ashiq | Br5 | Farid | KHA | NOO | Ugan8 | V68-43 | V72-I-148 | V73-I-157 | V75-I-27 | |
| 2/63 | Aslam | Br7 | Hart | KIB | Nur | V66-22 | V68-8 | V72-I-211 | V73-I-178 | V75-I-38 | |
| 22/62 | BAN | Br8 | IMR | Kim | ODO | V66-23 | V69-101 | V72-I-245 | V73-I-275 | WAT | |
| 25/62 | BED | BRM | Iran 9874 | KOM | PLU | V66-48 | V69-220 | V72-I-35 | V73-I-42 | WIN | |

70 strains used to completion

65 strains have additional vials

In 2012 a pilot study from nucleic acids extracted from convalescent scab materials, and egg-grown virus stored in the repository through 2011 demonstrated the ability to assemble significant variola virus genome sequence information, using Illumina sequencing without virus propagation, from some of this archival material. Of 10 archival stocks, 3 nucleic acid extracts were successfully processed to generate meaningful sequence. Virus sequence from some scab specimens was also assembled. The 7 others were further evaluated in 2013, and coverage was still minimal; this may in part be due to low titres in these archival (largely clinical, not research materials), or may be due to sample processing. In 2013 DNA libraries for Illumina sequencing have been generated from 20 of the 135 nucleic-acid extracted archival chorioallantoic membrane (egg-) grown viruses. Nucleic acid extracts, without additional propagation, were made under the auspices of the approved protocol “Use of live variola virus to maintain and regenerate non-infectious variola-derived materials for diagnostic development support”. Preliminary data indicate the need to refine the current methodologies in order to obtain more complete genome coverage and better define genetic diversity.

Use of live variola virus for diagnostic development support

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

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

This protocol was last renewed in March 2013 and is valid through December 2013.

The ability to validate nucleic acid-based and protein-based diagnostic capacity is critical for early detection and recognition of smallpox should a bioterror event result in reintroduction. The consequences of false negatives or false positives will significantly affect our global public health system and response efforts. As newer platforms are developed and reviewed by the US Food and Drug Administration (FDA), “older” (vintage 1990-2000) nucleic acid diagnostic platforms are no longer being supported by some companies. As additional orthopoxvirus sequence becomes available, the orthopoxvirus species diagnostic specificity of some nucleic acid signatures erodes. Protein-based diagnostics, either virus particle detection or the ability to detect a species-specific serologic response can augment diagnostic availability. Maintenance of the variola DNA and variola antigen stocks at the WHO Collaborating Centre for Smallpox and other Poxvirus Infections is important for future diagnostic development and validation. Live variola virus will continue to be needed to evaluate assays that detect viral particles, such as antigen capture assays.

DNA diagnostics

In 2010 several strains of cowpox virus showed cross-reactivity to a previously validated variola-specific signature (VRL1). Identification and characterization of unique variola virus signature sequences have been assessed for specificity and sensitivity to variola virus. Assessment of sensitivity has used non-infectious variola virus nucleic acid from the DNA repository. Together with the United States Laboratory Response Network (LRN) Technical Review Committee and FDA, these assays have gone through three stages of review and are entering the final stages of validation using multicentre reproducibility studies. The FDA has reviewed the process and has been supportive of this as a thorough process to validate the use of these new assays in the LRN. In the final stages of external, multicentre laboratory review, the LRN will be working with CDC to evaluate these assays using plasmid-containing noncontiguous assay target fragments (<500 bp each) of variola virus nucleic acid.

Protein diagnostics

Since late 2011, studies have been continuing on monoclonal antibody characterization, viral antigen capture assays, protein microarray development and novel methods for high-throughput viral neutralization assays applicable to variola virus. During the past year, work has focused on standardizing virus preparations, re-subcloning promising variola-specific hybridomas, and bioinformatics-guided approaches to variola-specific monoclonal antibody design. To date, using standard hybridoma production approaches, 4 monoclonal antibodies have been identified, which have preferential or species-specific recognition of variola virus. Using informatics-driven approaches, up to 36 additional variola-specific candidates have been identified, which will be tested using a newer approach to hybridoma generation. Next

stages of this work will use gamma-irradiated and live preparations of variola virus, as well as other orthopoxviruses, to develop and validate the sensitivity and specificity of assays using these reagents. Initial experiments have identified a panel of peptides that show promise as an approach to diagnostically define a monkeypox-specific serologic response.

**Discovery of new antivirals for smallpox treatment and prevention.
Assessment of neutralizing activity of vaccinee blood sera using
live *variola virus*.**

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

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

The discovery of novel antivirals for treatment and prevention of smallpox remains important. During 2012-2013, the SRC VB VECTOR tested more than 100 novel, chemically synthesized compounds of the NIOCH series and those of the Samara series, in surrogate orthopoxviruses (vaccinia virus, cowpox virus, and ectromelia virus). Thirty compounds that showed greater antiviral effects were selected for further studies. In 2013 the antiviral effects of these chemical compounds against the Ind-3a variola virus strain were studied in vitro.

One of the new compounds investigated, NIOCH-268, showed high activity against variola virus in vitro and so may be a promising drug against orthopoxviruses.

In 2013 research was performed to assess (by neutralization using the Ind-3a variola virus strain in experiments in vitro) the level of anti-smallpox antibodies in the sera of individuals vaccinated with the novel vaccine Revax-BT and those immunized with the vaccines currently licensed in Russia for vaccine prevention of smallpox. This study involved the use of sera from 30 individuals.

The levels of antibody titres in the sera of vaccinated individuals measured by neutralization with variola virus and with vaccinia virus (strain L-IVP) did not significantly differ from each other. There was a significant increase in the geometric mean titre of anti-smallpox antibodies in the sera of re-vaccinated individuals compared with those not previously vaccinated.

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

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

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A number of animal models of systemic orthopoxvirus disease have been developed to evaluate various safer smallpox vaccines or therapeutics. Historically, laboratory research efforts have tested several animal species for susceptibility to variola virus, but thus far other primates are the only non-human animals to exhibit overt illness. However, in order to induce illness in primates, the required infectious dose is much greater than the dose required for a natural infection (1×10^8 to 1×10^9 variola virus virions). The discovery of a novel, more permissive small-animal model system could facilitate the development of next-generation, safer smallpox vaccines and therapeutics.

In general, analogous to variola virus, inbred mouse strains are relatively difficult to infect, and obtain symptomatic illness, with monkeypox virus. A recent study surveying a large panel of inbred mouse strains identified a strain (CAST/EiJ) that is highly susceptible to infection with monkeypox virus (Americo, J. L., B. Moss, P.L. Earl, 2010. Identification of Wild-Derived Inbred Mouse Strains Highly Susceptible to Monkeypox Virus Infection for Use as Small Animal Models. *J. Virol.* 84(16):8172-80). Unpublished data from the same lab suggest that CAST/EiJ mice are highly susceptible to a range of orthopoxviruses (the genus of poxvirus to which both variola and monkeypox viruses belong) at lower infectious doses than seen in other inbred mouse strains. The potential usefulness of a rodent challenge model using variola virus – supplied from inbred populations with minimal intrinsic variability, greater availability of specific immunologic reagents, and ease of animal handling – makes it of great interest to determine whether they are susceptible to disease.

In 2012 CAST/EiJ female mice, 8-9 weeks old, were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were cohoused in groups of 2-3 animals per ventilated cage in the High Containment Lab (BSL 4). Standard mouse husbandry practices were performed during the experiment in accordance with CDC Institutional Animal Care and Use Committee guidelines. In addition to mouse chow, all animals received treats as appetite monitors, as well as a plastic nests and other enrichment materials.

Groups of 5 mice were inoculated intranasally (IN) on day 0. The inoculum was diluted to 10 μ l (5 μ l per nostril) in phosphate-buffered saline using sucrose-cushion-purified Harper strain of variola virus to achieve 5 different viral doses (5×10^2 , 5×10^3 , 5×10^4 , 5×10^5 or 5×10^6 plaque-forming units). Three mice were mock infected using the equivalent volume of gamma-irradiated 5×10^5 virus, two were additionally mock infected with diluent. Daily observations of the animals food consumption, activity level, weight, rash, and general appearance were recorded. Clinical criteria were used to assess for euthanasia criteria. Under anaesthesia with 3-5% of isoflurane gas, oral, ocular, and anal swabs, temperatures and a complete skin exam were performed three times a week. At day 21 post infection the animals were humanely euthanized for necropsy. Animals exhibited clinical signs (nasal and oral oedema, weight

loss) and symptoms (reduced grooming, reduced activity) of illness. Signs and symptoms were more profound in animals challenged with higher viral inocula. No animals died from illness, and by day 21 all animals appeared clinically well. At 21 days, all animals challenged with 10^3 or greater pfu live virus seroconverted; not all animals challenged with 100 pfu seroconverted. Animals infected with higher doses of virus shed virus in oral secretions. At day 21, evidence of systemic spread of virus, detected by polymerase chain reaction, was found in ovarian tissue in one animal challenged with 10^4 pfu variola-Harper.

Younger cast Eij mice, 5-6 weeks old, reportedly more susceptible to symptomatic and severe orthopoxvirus illness, will be challenged with variola virus Harper in November 2013 to determine whether animals in this age group are more susceptible to disease.

Use of live *variola virus* to evaluate antiviral agents against smallpox

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

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Additional external collaborators: John Connor; Randall Lanier.

This protocol was last renewed in March 2013 and is valid through December 2013.

The primary objective of smallpox bioterrorism preparedness is to save lives should smallpox somehow re-emerge. Thus, the development of antiviral strategies may be important in outbreak response efforts as well as in disease treatment. Current considerations have suggested the need for two antiviral compounds, with discrete mechanisms of action, to be licensed and available for use. Considerable progress has been made on advanced development of two compounds. One compound, ST-246, has been procured by the US government and is available for use through an investigational new drug protocol via the US strategic national stockpile. The Advisory Committee has continued to support the evaluation of new compounds, given the uncertainties of the drug development process. This project focused specifically on evaluation of antiviral efficacy, or mechanism of action, against live variola virus. Compounds specifically targeting viral proteins, viral processes, or cellular functions required by the virus but non-essential for the human host are presently of great interest. Critical steps to evaluate such therapeutics require *in vitro* and/or *in vivo* animal model characterization of their activity against live variola virus infection.

The manuscript by Dower, et al. [Identification of a Pyridopyrimidinone Inhibitor of Orthopoxviruses from a Diversity-Oriented Synthesis Library. *J Virol.* 2012 Mar;86 (5):2632-40.] recently identified CMLDBU6128 as an inhibitor of vaccinia, cowpox, and monkeypox viruses replication. The compound {IC₅₀ ~ 5 µM against vaccinia virus} appears to inhibit the poxviral DNA-dependent RNA polymerase. This polymerase is a core component of many poxviruses and is highly conserved between vaccinia, cowpox, monkeypox, and variola viruses. Though CMLDBU6128 targets the viral polymerase, it is not functioning as a nucleotide analog, as *in vitro* assays show that CMLDBU6128 directly inhibits RNA synthesis of intermediate and late transcription. This demonstrates that the mechanism of action of this drug is different from that of other antipox viral compounds. A transcription inhibitor may be of significant therapeutic benefit to inhibit expression of poxvirus immunomodulatory proteins.

This presentation will briefly update work on the evaluation of pyridopyrimidinone inhibitory compounds for activity against variola virus, and work to demonstrate the EC₅₀ of CMX-001 (the orally available derivative of Cidofovir) against strains of variola virus with various non-synonymous coding changes in the viral drug target, the DNA polymerase.

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

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

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

This protocol was last renewed in March 2013 and is valid through December 2013.

With the cessation of routine vaccinia virus vaccination following the declaration of global smallpox eradication in 1980, a significant portion of the global population has been left vulnerable to variola virus, the etiologic agent of smallpox. Thus, concerns have been raised regarding the potential impact of an outbreak of variola virus in an increasingly vulnerable population. Moreover, incidences of the closely related orthopoxvirus family member monkeypox virus have also been increasing. Thus, the significant health and economic consequences associated with an orthopoxvirus outbreak highlight the importance of novel antiviral therapeutic strategies. However, there is little information about the molecular mechanisms through which variola virus is able to modulate or subvert the host immune response. Further, it has been demonstrated that closely related orthopoxvirus species differentially modulate host cell responses and there are differences in the immunomodulatory proteins expressed by monkeypox virus and variola virus. Thus, this project focuses specifically on the identification of novel host targets for therapeutic intervention through the characterization of the functional host signalling networks targeted by variola virus.

There is an increasing appreciation that many host responses are regulated by kinase-mediated phosphorylation events independent of changes in transcription or translation. Thus, global investigations of the activation state of host kinases, i.e. the kinome, provide a functional mechanism for identifying host signaling networks or individual kinases that are altered during the course of infection. Several studies have demonstrated that pharmacological targeting of cellular processes may inhibit variola virus multiplication and enable prophylaxis. This research proposal uses global kinome analysis to identify host therapeutic targets and provide novel information about the molecular mechanisms of variola virus pathogenesis. Identification of novel host therapeutic targets may allow “repurposing” of drugs that are currently cleared by the US Food and Drug Administration for other indications, in the treatment of orthopoxvirus infection. Greater understanding of how variola virus modulates the cellular environment will also be critical to identify which other orthopoxvirus infection provides the best surrogate system. The information will assist in better characterization of animal models of systemic orthopoxvirus disease and their relation to smallpox disease progression – in particular, enhancement or modification of the non-human primate model system of smallpox disease.

This presentation will briefly update the results of our kinome analyses with particular focus on the differential modulation of host cell signaling responses following variola virus or

monkeypox virus infection. We will discuss our increased capacity for bioinformatics analysis, including the identification of the specific phosphorylation events that differentiated the monkeypox virus and variola virus kinome data. In addition, we will discuss our continued bioinformatics analysis of functional host cell signalling networks from the variola virus kinome data sets. Finally, our preliminary biological validation data (western blots and kinase inhibitors) for both viruses will be described.

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

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

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Additional external collaborators: Ichiro Kurane.

This protocol was last renewed in March 2013 and is valid through December 2013.

Variola virus neutralization in vitro remains an informative surrogate measure of smallpox vaccine efficacy. Our previous studies, using sera from vaccinia virus-vaccinees, have indicated that neutralization endpoint titres may differ between target viruses (variola virus - heterologous target versus vaccinia virus - homologous target). Slight differences in orthopoxvirus antigenic makeup likely account for these differences. The plaque reduction neutralization test (PRNT), which measures the ability of immune sera to neutralize mature virus forms (MV), has been used as a primary endpoint for the evaluation of vaccines. However, the ability of a vaccinee's sera to neutralize the extracellular enveloped virus (EEV) form of virus may be critical for vaccine efficacy as EEV is important for viral dissemination and ultimate disease pathogenesis [Smith et al. *J. Gen. Virol.* 2002, 83: 2915-31].

The development of new vaccines has included significant focus on the use of attenuated vaccine strains, such as Modified Vaccinia Ankara (MVA) and Lc16m8. These “third-generation” vaccines, however, were never tested directly for efficacy against smallpox during the eradication campaign since most were developed towards the end of that era. Evaluation of the ability of sera, generated through animal or human trials with less reactogenic smallpox vaccines, to neutralize MV and EEV forms of variola virus, will provide a measure of efficacy. The role of variola virus neutralization as a marker for vaccine efficacy is valuable for the evaluation of vaccines that do not elicit a “take”, the traditional measure of vaccine success.

This presentation will update results from collaboration with Japanese government and industry scientists to evaluate Lc16m8 vaccination regimens for variola virus MV neutralization. In particular, the neutralizing capacity of vaccinee sera will be compared between different orthopoxviruses (vaccinia virus versus variola virus), different strains of variola virus (representing both primary clades), and different vaccines (Dryvax versus LC16m8). We have also made significant progress on optimization of a higher throughput neutralization assay. The standardization of this neutralization assay with variola virus will allow a larger number of sera dilutions to be tested while using a smaller sample volume. This assay may be instrumental in evaluation of the ability of future MVA vaccinees' sera to neutralize variola virus, as a secondary endpoint for regulatory approval.

Progress towards development, approval and deployment of Arestvyr (ST-246)

Dennis E. Hruby

SIGA Technologies Inc., Corvallis, Oregon, United States of America

Arestvyr is a smallpox antiviral drug that is in late-stage development for use as a therapeutic agent for symptomatic patients, prophylactic agent in infected but non-symptomatic patients, and for concomitant administration with smallpox vaccines to improve their safety profiles. Arestvyr is an egress inhibitor that prevents the formation of the extracellular enveloped virus forms of orthopoxviruses. It has demonstrated excellent post-exposure efficacy in a large number of animal species, which included mouse, ground squirrel, prairie dog, rabbit, and non-human primates challenged with a variety of different pathogenic orthopoxviruses, including vaccinia, cowpox, rabbitpox, ectromelia, monkeypox and variola. In clinical studies, the drug appears to be safe and well tolerated. Once a day oral dosing provides blood exposure at or above levels that have been shown to be protective in animal studies. Following robust and iterative discussions with regulatory authorities, a clear and achievable pathway has been mapped out for the approval of the drug for use as a therapeutic agent for symptomatic patients, and SIGA and its federal partners are in the process of executing this plan.

Based on the data obtained to date, the US government has awarded a contract to SIGA for the acquisition of two million courses of Arestvyr to be added to the US Strategic National Stockpile. SIGA has executed on this contract and to date has deposited more than 500,000 treatment courses in the SNS and expects to complete this initial order by the third quarter of 2014. Work continues on the development of additional Arestvyr formulations and towards approval of the drug for additional indications.

European Medicines Agency approves the non-replicating smallpox vaccine IMVANEX (IMVAMUNE)

Lars Staal Wegner

Bavarian Nordic, Denmark

The European Commission has granted marketing authorization for IMVANEX (MVA-BN) for active immunization against smallpox disease for the general adult population, including people with weakened immune systems (people infected with HIV or diagnosed with atopic dermatitis). The authorization covers all 28 European Union Member States and European Economic Area countries Iceland, Liechtenstein and Norway.

IMVANEX (IMVAMUNE in the USA) is a live, highly attenuated vaccinia strain vaccine that does not replicate in human cells and has been developed as a stand-alone smallpox vaccine. IMVANEX has been tested in more than 7000 subjects, including 1000 subjects from risk groups with contraindications for conventional smallpox vaccines, i.e. HIV-infected and atopic dermatitis patients. IMVANEX, has in 19 completed or ongoing clinical trials, demonstrated a favourable safety profile in healthy individuals as well as in populations with impaired immune function.

IMVAMUNE is currently part of the US Strategic National Stockpile of medical products, and other countries are also implementing IMVANEX in their preparedness. In 2012, the US government expanded the population that is eligible to receive IMVAMUNE during an emergency. In the event of a smallpox public health emergency, the government may now authorize the use of IMVAMUNE to protect individuals of all ages with HIV infection or atopic dermatitis, including children, pregnant women, and nursing mothers with these conditions.

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

Update on recent studies of the third-generation smallpox vaccine LC16m8

Hiroyuki Yokote

The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan

In the 1970s, an attenuated replication-competent vaccinia virus, LC16m8, was developed from the Lister strain by serial passaging in primary rabbit kidney cells. LC16m8 has demonstrated low neuro-virulence and protective efficacy in animal models. The LC16m8 vaccine has been given so far to approximately 90,000 infants and more 8,000 members of the armed forces without any severe adverse effects. Based on the clinical data obtained during its development stage, the LC16m8 vaccine was licensed in Japan in 1975.

Subsequently, however, vaccination against smallpox ceased in 1976 in Japan. In response to recent global concerns over the possible use of smallpox virus as an agent of bioterrorism, manufacturing of the LC16m8 vaccine was resumed in Japan and the vaccine has been being stockpiled since 2001. Currently, LC16m8 is given once to populations at high risk for smallpox, such as members of the armed forces in Japan, while it is also recommended for such groups to be revaccinated periodically. Taking the current situation into consideration, we conducted research studies on revaccination in cases involving LC16m8.

Results showed that an effective booster response was confirmed in subjects given LC16m8 who had previously received one or more smallpox vaccinations. In addition, the data obtained in animal studies indicated that LC16m8 alleviated skin lesions that were often seen in subjects vaccinated with a conventional smallpox vaccine. LC16m8 also showed a booster effect comparable to that of conventional vaccines. We also obtained data in animals showing that a booster vaccination with LC16m8 elicited an immune response equivalent to that of conventional vaccine strains.

All these data support the conclusion that LC16m8 is an adequate vaccine to be stockpiled for preparedness against possible smallpox attacks. LC16m8 can be expected to work effectively when used alone as well as in combination use with first- and second-generation vaccines.

Update on the development of Brincidofovir (CMX001) for smallpox and other indications

Lawrence C. Trost

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Brincidofovir (CMX001) is a potent inhibitor of multiple double-stranded DNA (dsDNA) viruses, including adenovirus, cytomegalovirus (CMV) and variola major. The key attributes of brincidofovir compared with intravenously administered cidofovir (Vistide) are improved ease of use, oral bioavailability, increased potency and decreased nephrotoxicity. In addition, brincidofovir has demonstrated a favourable resistance profile and a low risk of significant drug-drug interactions.

It has been administered to more than 900 human subjects, many with life-threatening illnesses caused by dsDNA viruses. It has also been studied in patients with renal or hepatic impairment and paediatric subjects as young as 1 month of age. A Phase 3 study for prevention of CMV in immunocompromised transplant patients is currently enrolling with doses that, scaling from efficacious doses in animal models, are also proposed for treatment of smallpox.

The US FDA has agreed that the intradermal rabbitpox model in rabbits and the intranasal ectromelia model in mice are acceptable animal models to support development of brincidofovir for a therapeutic indication for treatment of smallpox. The activity of brincidofovir was recently demonstrated in a blinded efficacy study in the rabbitpox model in which a statistically significant survival benefit of two different regimens was observed when treatment was initiated after clinical signs of infection.

In the first half of 2013, the Biomedical Advanced Research and Development Authority (BARDA) notified Chimerix of its intention to exercise an option to provide additional funds (Option Segment 1) to further advance the development of brincidofovir for treatment of smallpox. Supported by the work of BARDA's Animal Model Development Program, Chimerix will initiate pharmacokinetic studies of brincidofovir in the rabbitpox and ectromelia models in the last quarter of 2013. These studies will support dose selection for pivotal efficacy studies. Brincidofovir is expected to be commercially available in tablet and liquid formulations. Manufacturing of brincidofovir has been validated at commercial scale and the drug is stable for years. Taken together, the smallpox animal model and human efficacy data against related viruses support the likely efficacy of brincidofovir for treatment of smallpox.

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

US Food and Drug Administration progress on facilitating the development and approval of 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 MCMs—with the goal of achieving FDA approval³—as well as facilitating timely access to MCMs in the event of a public health emergency.

The US government is supporting the development of smallpox MCMs, including drugs, vaccines, and diagnostic tests. This presentation highlights the regulatory progress made since the 2012 ACVVR meeting.

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

Regulatory uncertainties related to smallpox MCMs reflect scientific uncertainties. Diagnostics, drugs, and vaccines each present their own unique set of scientific uncertainties. FDA is working very closely with MCM developers—through mechanisms such as interactive review—to guide the development of smallpox MCMs and establish feasible and appropriate regulatory pathways for their approval. The focus of FDA's interactions with MCM developers since the 2012 ACVVR meeting has been on providing feedback on proposed studies to support safety, pharmacokinetic and animal model efficacy studies for antiviral drugs and pivotal efficacy studies for the attenuated vaccine. Measurable progress has been made and smallpox MCMs continue to advance in development.

³ 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.

European Medicines Agency activities on smallpox

Robin Ruepp

European Medicines Agency, London, United Kingdom

The European Medicines Agency (EMA) is the European Union (EU) agency responsible for the protection of public and animal health through the scientific evaluation and supervision of medicines. The EMA has published a note for guidance on the development of vaccinia virus based vaccines against smallpox (CPMP/1100/02). In addition, a guidance document on the use of medicinal products for treatment and prophylaxis of biological agents (including smallpox), is available (CPMP/4048/01). Several tools are available during preauthorisation for products under development, such as Scientific Advice, Orphan designation based on Regulation (EC) No 141/2000 and Paediatric investigation plans, which are mandatory requirement under the paediatric legislation (Regulations (EC) No 1901 and 1902/2006).

Tecovirimat received in 2012 a positive orphan designation for the treatment of cowpox infection, as the condition affects fewer than 0.001 persons in 10,000 in the EU, which is below the threshold for orphan designation (5 in 10,000). Orphan status cannot be given for therapies or vaccines against smallpox, as it has a zero incidence within the EU.

For Imvanex a paediatric investigation plan is in place, which targets the following indication: active immunisation against smallpox infection and disease in the paediatric population, including HIV patients and patients with atopic dermatitis. Besides requesting extrapolations of available study results to the paediatric population, the requirement to gather data in children is deferred until the time of a potential outbreak of smallpox. For initial marketing authorisations, the EMA's scientific committee for medicinal products for human use (CHMP), provides scientific opinions to the European Commission as one of its tasks. Imvanex received a positive Commission Decision for a Marketing Authorisation (MA) under exceptional circumstances on 31 July 2013 based on a positive opinion of the CHMP by majority on 30 May 2013 for the indication "Active immunisation against smallpox in adults".

A marketing authorisation under exceptional circumstances (Article 14(8) of Regulation (EC) No 726/2004) means that, according to the present state of scientific knowledge, comprehensive information on the efficacy and safety of the medicinal product under normal conditions of use cannot be provided, which justifies making the requested authorisation subject to an annual reassessment and on the conduct of certain requirements.

The CHMP concluded that Imvanex's benefits are greater than its risks following the assessment of five main studies involving healthy and immunocompromised individuals. The studies showed that Imvanex was effective at stimulating an immune response against MVA-BN when used as primary or as booster vaccination. However, owing to the prevalence of the condition and the fact that clinical efficacy trials cannot be carried out, comprehensive data on the efficacy and safety were not available at the time of the assessment and the CHMP therefore recommended that Imvanex be approved in the EU under exceptional circumstances. For Imvanex effectiveness data were requested to be gathered in observational prospective non-interventional cohort studies in case of re-emergence of circulating smallpox. In addition, among other studies, post-authorisation studies will be conducted in

children at the time of a potential outbreak of smallpox to provide safety and effectiveness data in this population.

WHO orthopoxviruses laboratory network for early smallpox diagnostics

Jean-Claude Piffaretti

Interlifescience, Massagno, Switzerland

The laboratory network

In 2012 the document “WHO Laboratory Network for Early Smallpox Diagnostics: Terms of References and Criteria” was produced in collaboration with the WHO Smallpox Secretariat. This document clearly indicates the tasks expected from a candidate laboratory and the technical and quality criteria that be fulfilled. In order to identify appropriate potential laboratories, the document was sent by the WHO Smallpox Secretariat to WHO Regional Offices. The process is still in progress.

Molecular diagnostic methodologies

An ad hoc Independent Technical Group has been formed to propose a standard diagnostic methodology for orthopoxvirus molecular diagnostics, as well as for variola virus molecular identification. The group is formed by representatives of the two WHO Collaborating Centres (CDC and VECTOR), by experts from Germany, India, Brazil, India, South Africa, and the WHO Smallpox Secretariat.

The ad hoc Independent Technical Group agreed on the following points:

1. The assay to be proposed is for clinical specimens only.
2. The methodology will be based on real-time PCR with hybridisation probes or TaqMan-based probes.
3. The performance of the assay(s) should have been validated with the most frequent orthopoxviruses infecting humans by an independent group of laboratory experts.
4. The appropriate controls should be provided (positive, negative, human DNA, inhibitory, etc.).
5. The assays reagents, including the controls, should be produced under good manufacturing practice conditions. A kit format would be optimal. Each production lot of assay should be monitored for quality.
6. The assay scheme should be based on:
 - a. a first step (step A) allowing the identification of the most frequent orthopoxviruses infecting humans, including variola virus,
 - b. a second step (step B) allowing specific identification of variola virus (one-two assays).

7. In any case, an uncertain or positive result must be confirmed by the two WHO Collaborating Centres.
8. Several methods published by Olsen et al. (2004) are considered appropriate for step A. Assays for step B will be discussed later.
9. The group is now reviewing the options that are available.

Agenda for 2013-2014: finalize the proposal for a molecular diagnostic and establish the procedures to be applied.

Annex 2

Agenda

15th Meeting of the WHO Advisory Committee on Variola Virus Research

24–25 September 2013

**Executive Boardroom, WHO Headquarters
Geneva, Switzerland**

24 September 2013

- 9:00 - 9:15 Opening – Dr K. Fukuda, Assistant Director-General, Health Security and Environment
Election of Chair
- Variola virus reports**
- 9:15 – 9:30 Report of the Secretariat - WHO secretariat – A. Costa
- 9:30 – 9:45 Update on research proposals submitted to WHO 2012/2013 and recommended for approval – R. Drillien
- 9:45 – 9:55 Report on the variola virus collection at the WHO Collaborating Centre Repository in VECTOR, Koltsovo, Novosibirsk, Russian Federation – A. Sergeev
- 9:55 – 10:05 Report on the variola virus collection at the WHO Collaborating Centre for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA – I. Damon
- Variola virus research 2012 – 2013 Update**
- 10:05 – 10:35 Use of live variola virus for diagnostic development support – I. Damon
- 10:35 – 11:00 **Tea/Coffee Break**
- 11:00 – 11:30 Discovery of new antiviral for smallpox treatment and prevention/
Assessment of the neutralizing activity of vaccine blood sera using live variola virus – L. Bulychev
- 11:30 – 12:00 Use of live variola virus to determine whether CAST/Eij mice are a suitable animal model for human smallpox – I. Damon

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- 12:00 – 12:30 Use of live variola virus to evaluate antiviral agents against smallpox – V. Olson
- 12:30 – 14:00 **Lunch**
- 14:00 – 14:20 Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention – J. Kindrachuk
- 14:20 – 14:40 Use of live variola virus to support less reactogenic vaccine development: continued evaluation of third-generation vaccines – V. Olson
- 14:40 – 15:00 Progress towards approval and deployment of Arestvyr® (ST-246) – D. Hruby
- 15:00 – 15:20 European Medicines Agency (EMA) approves the non-replicating smallpox vaccine IMVANEX® (IMVAMUNE®) - L. Wegner
- 15:20 – 15:50 **Tea/coffee Break**
- 15:50 – 16:10 Update on the recent findings of third-generation smallpox vaccine LC16m8 – H. Yokote
- 16:10 – 16:30 Update on the development of Brincidofovir (CMX001) for smallpox and other indications – L. Trost
- 16:30 – 16:50 US Food and Drug Administration (FDA) progress on facilitating the development and approval of smallpox medical countermeasures – L. Borio
- 16:50 – 17:10 EMA activities on smallpox – R. Ruepp
- 17:10 – 17:30 WHO orthopoxviruses laboratory network for early smallpox diagnostics – J.C. Piffaretti
- 17:30 – 18:30 General discussion
- 18:30 – 19:30 **Social event**

25 September 2013

- 9:00 – 9:20 WHO Smallpox Vaccines - WHO Secretariat – A. Costa
- 9:20 – 9:50 Variola virus repositories biosafety inspection visits 2012 and 2014 – N. Previsani
- 9:50 – 10:30 General discussion and preparation of draft meeting report
- 10:30 – 11:00 **Tea/Coffee Break**
- 11:30 – 12:30 General discussion and preparation of draft meeting report (continued)

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- 12:30 – 13:30 **Lunch**
- 13:30 – 15:00 General discussion and preparation of draft meeting report (continued)
- 15:00 – 15:30 **Tea/Coffee Break**
- 15:30 – 16:30 Final discussion and finalization of draft report

CLOSE OF ACVVR MEETING

Annex 3

List of Participants

15th Meeting of the WHO Advisory Committee on Variola Virus
Research

24–25 September 2013

Executive Boardroom, WHO Headquarters
Geneva, Switzerland

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