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WHO Advisory Committee on Variola Virus Research

Report of the Seventeenth Meeting

Geneva, Switzerland

12–13 January 2016

PANDEMIC AND EPIDEMIC DISEASES

World Health Organization

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Executive Summary

The Advisory Committee on Variola Virus Research held it Seventeenth meeting on 12 and 13 January 2016 at WHO headquarters in Geneva.

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

It was also provided with updates on the continuing research projects using live variola virus for the development of diagnostic tests, animal models, smallpox vaccines, and antiviral and therapeutic agents. As concluded in the Fifteenth meeting of the Advisory Committee (24 and 25 September 2013), the only new projects to be approved were for antiviral agents against smallpox. The Advisory Committee discussed the estimated timelines for the ongoing research projects and expected that the completion and final review of these projects will take a minimum of three years. Of the proposals for research submitted in 2014-2015 that involved live variola virus the Advisory Committee's Scientific Subcommittee approved three from Vector (all extensions of existing projects) and five from CDC (four extensions and one new project).

Participants from CDC reported advances in the area of diagnostics, including the preparation and use of monoclonal antibody mixtures in diagnostic assays for viral particles or antigen capture, and variola virus proteome chips for profiling antibody responses. Work is also under way to investigate immune responses including duration of immune memory and viral neutralization. At Vector, an investigational new live smallpox vaccine was shown to induce neutralizing activity equivalent to that induced by conventional vaccines.

Progress was reported on the development of tecovirimat, one million treatment courses of which have been added to the US Strategic National Stockpile. Additional human safety studies are planned. Studies of brincidofovir, which is also in development for the treatment of cytomegalovirus disease and is active against variola virus in vitro, continue in two animal models.

Work has continued on the discovery and development of antiviral agents against variola virus. At Vector the lead compound continues to be NIOCH-14, an analogue of tecovirimat, but another unrelated compound has shown promising activity against orthopoxviruses. CDC researchers continue to look at agents that act at different stages of virus infection.

With regard to antiviral agents that are active against variola virus in vitro and in animal experiments, the Advisory Committee further received reports from two regulatory agencies, the US Food and Drug Administration and the European Medicines Agency, on progress towards completing the studies required for licensure for the treatment of smallpox in humans. The two regulatory agencies informed the Advisory Committee that protocols to test the effectiveness and safety of therapeutics in the field at the time of outbreaks must also be developed as a prerequisite for potential licensure of those agents.

New animal models, including outbred ICR mice and humanized mice, are being examined for their value in smallpox research. Regulatory authorities have engaged in discussions with researchers and pharmaceutical companies about the acceptability of these models and their results.

The Advisory Committee also received reports on the continued evaluation of third-generation vaccines, clinical trials to study them, and updates on LC16m8, including post-marketing surveillance, and Imvamune®/Imvanex®, which is being added to the Strategic National Stockpile in the USA.

The WHO Secretariat reported on the biosafety inspection visits carried out at the two smallpox repositories (Vector in December 2014 and CDC in May 2015) and the next planned round of inspections. A brief update was also provided on the smallpox vaccine stockpile held by WHO.

The Advisory Committee received a report from the Chair of the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology related to Smallpox which concluded that the nature of the risk of re-emergence of smallpox has changed significantly and is evolving. The Advisory Committee responded to the issues raised in the report regarding the implications for variola virus research as follows:

- The Advisory Committee recognized the need for increased preparedness to deal with the potential consequences of the synthesis and possible re-emergence of variola virus and encouraged the expansion of expertise in the area of laboratory biosafety and biosecurity and diagnostics for this purpose. It also recommended increased capacity of staff trained to work with dangerous pathogens and to recognize smallpox cases.
- The Advisory Committee concluded that there was no need to increase the number of sites where research using live variola virus could be undertaken beyond the two existing authorized global repositories, based on their assessment of the risk versus benefit. However, it recommended that more laboratories around the world should develop capacity for smallpox diagnostics which did not need live variola virus. The Advisory Committee also recommended that point-of-care diagnostic tests for variola virus infection should developed; their use should be coordinated to assure accuracy of testing in low disease prevalence situations.
- Given the change in the risk of re-emergence of smallpox due to synthetic biology technology, the Advisory Committee reviewed its terms of reference and concluded that they were broad enough to include the area of synthetic biology technology if needed. There was general agreement that the areas of current research – focusing on translational outcomes for diagnostics, therapeutics and vaccines – remain important.
- However, the Advisory Committee agreed that additional members with appropriate expertise related to new technologies, such as synthetic biology, would be welcome. The Advisory Committee gave special attention in its review of the current research agenda to assess whether there are or will be additional needs for smallpox control measures in case of re-emergence of a synthesized and/or modified variola virus.
- Finally, as recommended by the Independent Advisory Group on the Public Health Implications of Synthetic Biology Technology Related to Smallpox, the Advisory Committee revised the *WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA*. Some recommendations have been expanded to include clarification on compliance with the guidelines and on the limit of 500 base pairs for synthesis of DNA fragments applied to diagnostics. A definition of the term "variola virus DNA" has been added. The Advisory Committee specified that permission to express one or more variola virus genes must be sought from the WHO Secretariat and has outlined clear reporting requirements. The Advisory Committee further strongly recommended that the revised recommendations be widely distributed and adopted by Member States as part of their national biosafety regulations.

1. Report of the WHO Secretariat - Dr R. Bruce Aylward and Mr A. Costa

The WHO Advisory Committee on Variola Virus Research met on 12 and 13 January 2016 under the chairmanship of Professor G. Smith with Mr D. FitzSimons as Rapporteur.

1.1 Dr R. Bruce Aylward, the Executive Director ad interim Outbreaks and Health Emergencies and Special Representative of the Director-General for the Ebola Response, opened the meeting. He praised the Committee for its continued productivity over many years and its contributions to the world's better preparedness with countermeasures in case of release of variola virus or the re-emergence of smallpox. He recalled that the Committee's objectives in advising the Director-General were: to review proposals for research with live variola virus; to identify and prioritize future research needs; to determine implications for the storage of variola virus stocks; and to provide advice on the revision of guidelines for the distribution and handling of sequences of variola virus DNA and other viral material. An item on "Smallpox eradication: destruction of variola virus stocks" is on the provisional agenda of the 138th session of the Executive Board[1] (25-30 January 2016), in preparation for further discussion at the Sixty-ninth World Health Assembly in May 2016.

1.2 At the request of the Health Assembly in 2014, the WHO Secretariat convened two meetings in 2015 on synthetic biology and the implications for the risk of the creation of variola virus (see paragraph 2.1 below). The report of the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology related to Smallpox, which met on 29 and 30 June 2015, has been published on the WHO website.[2] In preparation for the Independent Advisory Group meeting, a Scientific Working Group on Synthetic Biology and Variola Virus and Smallpox was convened on 16 and 17 April 2015 to review the evidence and inform the deliberations of the Independent Advisory Group; their report is annexed to the Independent Advisory Group's report. The Independent Advisory Group was tasked with providing guidance to the Director-General on the implications of synthetic biology technology as it relates to public health measures for preparedness and control for a potential re-emergence of smallpox.

1.3 Dr Aylward conveyed to the Advisory Committee that the Director-General needed their views on reasonable time lines for research currently being undertaken and on criteria that would determine those limits. There were as yet no licensed antiviral agents for use against smallpox although two were close to licensure, but vaccines and diagnostics are licensed. The historic time scale for research and development of any new antiviral agents is estimated to be about 10 years. Accelerating this process needed definition of the characteristics of what would satisfy regulatory

[1] See document EB138/22 (http://apps.who.int/gb/ebwha/pdf_files/EB138/B138_22-en.pdf, accessed 17 January 2016).
[2] Document WHO/HSE/PED/2015.1 (http://apps.who.int/iris/bitstream/10665/198357/1/WHO_HSE_PED_2015.1_eng.pdf, accessed 15 January 2016).

authorities. Objective criteria were needed for discussions and decisions about retention of the stocks of live variola virus.

1.4 Mr A. Costa gave an overview of the WHO Secretariat's activities in the period December 2014 to the end of 2015, including the review of the research proposals submitted by the State Research Center of Virology and Biotechnology "Vector"(Vector) in the Russian Federation and the Centers for Disease Control and Prevention (CDC) in the United States of America (USA) (see section 3 below). The Secretariat organized the biosafety inspections of these two repositories of variola virus, which took place in December 2014 (Vector) and May 2015 (CDC). The draft reports of the results of these inspections were shared with the repositories for comment in December 2015 and are expected to be finalized and published shortly.

1.5 The recently discovered old vials containing variola virus in the US National Institutes of Health were destroyed in February 2015 in the presence of a WHO team.

1.6 A consultation on WHO prequalification of vaccines with Member States that hold smallpox global vaccine stockpiles was held in September 2015. As mentioned above, the Secretariat also convened meetings of the Scientific Working Group on Synthetic Biology Technology (Geneva, 16 and 17 April 2015) and the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology related to Smallpox (Geneva, 29 and 30 June 2015).3

1.7 In discussion, Mr Costa confirmed that 35 million doses of smallpox vaccine had been pledged to the WHO global stockpile and that WHO physically held in safe storage some 2.5 million doses. Questions were raised about the expiry date of stored vaccines, although it was noted that all potency tests on stored material confirmed that freeze-dried vaccines remained remarkably stable and active long after expiry with optimal storage conditions.

2. Report on the consultation on synthetic biology - Dr M. Sprenger

2.1 Dr M. Sprenger, Chair of the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology related to Smallpox, which met in Geneva on 29 and 30 June 2015, presented a summary of the Group's deliberations. Its meeting had been preceded by a meeting of a Scientific Working Group on Synthetic Biology and Variola Virus and Smallpox (Geneva, 16 and 17 April 2015) and the outcome of the two meetings and the report had been submitted to the Director-General. He described the public health implications of synthetic biology technology in terms of the

[3] The report of the Scientific Working Group on Synthetic Biology Technology is contained within that of the Independent Advisory Group (http://www.who.int/csr/resources/publications/smallpox/synthetic-biology-technology-smallpox/en, accessed 15 January 2016).

risk of smallpox, preparedness and research, all within the context of the World Health Assembly's resolution adopted in 1996 to destroy the stocks of variola virus held in the two authorized repositories.

2.2 The Independent Advisory Group noted the rapid advances in synthetic biology and the increasing ease of obtaining genetic material and machines for their assembly. The Group's major conclusion was that henceforth there will always be the potential to recreate variola virus and therefore the risk of smallpox re-emerging can never be eradicated. The change in risk has raised important questions about whether the world is prepared and what steps are needed for preparedness against re-emergence of smallpox, the implications for public health preparedness, and research related to this new risk. The question was whether the new, additional risk changes the parameters of the discussion for the destruction of the variola virus stocks; if so, that has implications for research. These include:

- review of the terms of reference of the Advisory Committee to take account of the new situation, potentially being expanded and more specific
- the importance of adapting the rules and regulations for manipulating and synthesizing variola virus genes
- consideration of future research needs, including the number of research sites, and development of expertise at global level
- the need for public health laws to reinforce WHO's recommendations concerning the distribution, handling and synthesis of variola virus DNA.

2.3 In discussion, Dr Sprenger stressed that the stocks held in the two repositories were to be destroyed in accordance with the Health Assembly's decision in 1996, unless the Health Assembly revisited the decision. The situation nevertheless demanded increased preparedness, not just for smallpox but other epidemic diseases as well. He pointed out that re-creation of variola virus is complex and that a deliberate and sustained effort would be required to do so; and even though this has a low likelihood it could have a high impact. Developments in synthetic biology meant that it was critical to review the guidelines for distribution and handling of variola virus DNA fragments and viral material. An important point raised was that WHO's recommendations cannot be enforced unless they are enacted into the legislation of Member States with harmonization of that legislation between countries.

3. Update on research proposals submitted to WHO in 2014-2015 - Professor D. Evans

3.1 Professor D. Evans described the process by which the Advisory Committee's Scientific Subcommittee reviewed proposals submitted for research with live variola virus. During the period December 2014-September 2015 it had received eight proposals from CDC and five from Vector. Five of those from CDC were approved (including four extensions and one new project). Three proposals from Vector, for extension of existing projects, were approved. In some cases revisions had been requested, delaying the time to decision. (For details of the proposals and the Subcommittee's comments see Annex 1.)

3.2 Professor Evans, on behalf of the Scientific Subcommittee, commended the WHO Collaborating Centres' continued pursuit of a variola virus research agenda but, as a general observation, its members expressed concerns about the limited progress in many of the ongoing projects and their open-ended time frames. The request was made to ensure clear timelines and endpoints in proposals to the Advisory Committee. It was also noted that the most important criteria for approval of research by the Advisory Committee was that the proposed study had to be shown to be "essential for public health benefit". Studies that were attempting to screen a wide variety of new compounds without substantial evidence for their selection were unlikely to be approved.

3.3 In discussion, the Chair recalled that both the Advisory Committee and the Advisory Group of Independent Experts to review the smallpox research programme (AGIES) had made a recommendation that there was no need to work with live variola virus,[4] although the Advisory Committee had made an exception for discovery of antiviral agents as the two current candidates were in the pre-licensure phase.[5] As noted by the 67th World Health Assembly, [6] "the majority view" of the WHO Advisory Committee on Variola Virus Research at their fifteenth meeting "was that no need exists to retain live variola virus for development of further diagnostics for smallpox or for the development of safer smallpox vaccines beyond those studies already approved" and that of the Advisory Group of Independent Experts to review the smallpox research programme that had met in Geneva on 5-6 November 2013, to review research with live variola virus during the period 1999–2013 "concluded that there is no need, from a global public health perspective, to retain live variola virus for any further research."

[4] Report of the Advisory Group of Independent Experts on Smallpox, document WHO/HSE/GAR/BDP/2010.4 (http://apps.who.int/iris/bitstream/10665/70509/1/WHO_HSE_GAR_BDP_2010.4_eng.pdf, accessed 15 April 2015), respectively.
[5] Report of the fifteenth meeting of ACVVR, document WHO/PED/CED/2013.2 (http://www.leighainslie.com/who_other/WHO_HSE_PED_CED_2013-2.pdf, accessed 15 April 2016).
[6] Report by the Secretariat at the 67th WHA, Agenda Item 16.3 Smallpox eradication: destruction of variola virus stocks (A67/37: http://apps.who.int/gb/ebwha/pdf_files/WHA67/A67_37-en.pdf, accessed 18 April 2016)

3.4 Advisory Committee members noted that the range of potential antiviral candidate compounds and groups was broad, yet little progress had been made except for the two antivirals in the final stages of licensure. It was essential to first generate good data on potential compounds against other orthopoxviruses before moving on to work with live variola virus. Participants recognized the need for proposals for antiviral agent discovery to be clear about their rationale and pre-proposal work; that all research had to be time-limited and had to be considered essential for public health benefit.

4. Report on the variola virus collection at the WHO Collaborating Centre Repository in Vector, Koltsovo, Novosibirsk Region, Russian Federation7 - Dr A. Agafonov

4.1 Dr A. Agafonov confirmed that the work on the approved research projects with variola virus at the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository of Variola Virus Strains and DNA complied with national and international requirements. The variola virus collection still consisted on 120 strains from Africa, the Americas (Brazil), South-East Asia, Europe and the eastern Mediterranean. Of these, 32 were demonstrated to be viable. Strains were stored in freeze-dried and frozen cultures and as historic scab material from patients. Storage conditions were rigorous and secure, with limited and controlled access. The WHO inspection team in 2014 confirmed that the storage conditions complied with national and international requirements. In 2015, Ind-3a and Butler strains were grown in Vero cells and used in approved research on potential antiviral agents, assessment of neutralizing activity in sera from vaccinees, and the development of animal models for testing antiviral and prophylactic compounds. The research is planned to continue in 2016-2017 with approval from the Scientific Subcommittee, but no sequencing is being done.

5. Report on the variola virus collection at the WHO Collaborating Center for Smallpox and Other Orthopoxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America - Dr I. Damon

5.1 Dr I. Damon reported the status in 2015 of the collection held by CDC. The BSL-4 facility had been closed for maintenance in March-July 2015 and in May 2015 the WHO inspection took place. No viruses were added to or withdrawn from the long-term repositories. Working stocks of virus were grown and purified for research use and the removal of strains was inventoried between October 2014 and December 2015. The virus was used in reagent preparation for, and evaluation of, protein diagnostics, development of third-generation vaccines, investigation of humanized mice as animal models of smallpox, and evaluation of monoclonal antibodies as antiviral agents against variola virus. A cloned gene was provided to the Vanderbilt University Medical Center under a

[7] Abstracts of this and subsequent presentations are contained in Annex 1.

Materials Transfer Agreement. Data on genomic sequencing of variola virus, with fragments of about 300 base pairs, are collected and analysed at CDC. Of 135 samples processed, 42 complete sequences have been obtained; none were identical to existing materials. Work continues on improving the next generation sequencing of variola virus samples to continue improving the efficiency of direct sequencing.

5.2 In February 2015 a WHO team witnessed the destruction of old vials of variola virus found in the US National Institutes of Health (Bethesda campus).

5.3 In the discussion, it was noted that several stocks had been used to completion, that is to say, stocks were destroyed after experiments had been completed, and that no virus had been grown to master-seed stock.

6. Use of live variola virus to maintain and regenerate non-infectious variola-derived materials for diagnostic support - Dr I. Damon

6.1 Dr Damon presented an update on the development of nucleic acid-based and protein-based diagnostics. Variola virus-specific DNA diagnostic assays are progressing toward regulatory approval.

6.2 Monoclonal antibodies highly specific for variola virus have been generated in the traditional way (starting from immunization of BALB/c mice with irradiated variola virus, generating hybridomas, screening and selecting single clones) and by bioinformatic approaches (identification of variola virus-unique peptides on surface proteins for immunization of BALB/c mice and screening for monoclonal antibodies). Dr Damon described the generation, characterization, reactivity and cross-reactivity, and specificity of these monoclonal antibodies. New diagnostic assays using these antibodies may allow for rapid detection of variola virus particles/antigens (capture assay) and could supplement real-time PCR assays for species-specific detection and differentiation of orthopoxviruses with high sequence identity.

6.3 She further described work on the development of a variola virus proteome chip with the potential for differentiating antibody responses to vaccination from those following infection. Open reading frames encoding variola virus proteins were expressed, purified and printed on microarray chips. Preliminary data show that different variola antigens are recognized in smallpox convalescent sera compared with those in vaccinees' sera. In general, more intense variola antigen recognition is manifest in smallpox convalescent sera than in those from vaccines.

6.4 In discussion it was observed that the work on the proteome chip did not require the use of live variola virus. Estimates of time lines for development would be submitted. With regard to the DNA assays, questions were asked about the likely time until licensure, but it was pointed out that

their development had been delayed because of the advent of new technologies and the discovery of cross-reactivity with cowpoxvirus and possibly other orthopoxviruses, necessitating a new approach.

7. Discovery of new antivirals for smallpox treatment and prevention - Dr S. Shchelkunov

7.1 Dr S. Shchelkunov said that NIOCH-14, a structural analogue of tecovirimat, was being tested in pre-clinical trials in the Russian Federation. In 2014, some 120 newly synthesized chemical compounds of different classes were tested against surrogate orthopoxviruses. Ten were selected for further investigation in 2015 against variola virus in vitro; these included benzimidazoles, oxazolidines, indoles, pyrrolidines, oxoalkylamides and the triterpene betulin. Only one, a derivative of 3-amino-5-nitropyridine designated Sag18, showed relatively high anti-orthopoxviral activity. NIOCH-14 continues to be the lead compound for research.

7.2 The results indicate that besides tecovirimat and its analogue NIOCH-14 [and brincidofovir], no compound has been found to be highly active against variola virus and underlined the need to continue the search for different class of antiviral agents against orthopoxviruses.

8. Assessment of the neutralizing activity of vaccinated human volunteers' blood sera and those of immunized laboratory animals using live variola virus - Dr S. Shchelkunov

8.1 Dr Shchelkunov presented the results of studies on neutralizing antibodies in sera from humans immunized with a conventional smallpox vaccine and from rabbits immunized with an investigational bivalent oral recombinant live smallpox vaccine, Revax-BT. High levels of neutralizing antibodies against the Ind-3a strain of variola virus were found in the volunteers' sera 18 and 36 months after immunization. Similarly, sera from rabbits one month after immunization also had high levels of neutralizing antibodies against the same strain.

8.2 In discussion it was stated that test subjects should be followed up and re-tested every three years. The question of whether the presence of neutralizing antibodies equated with protection remained open, although the view was expressed that experience post-eradication of smallpox indicated that protection was most likely life-long.

9. Use of live variola virus to determine whether mice are a suitable animal model for human smallpox - Dr V. Olson

9.1 Dr V. Olson reported that experiments with CAST/Eij mice were not successful and ruled them out as a good model of smallpox.

9.2 Attention has now shifted to humanized mice, mouse models with humanized immune systems. Three strains have been recently studied to evaluate susceptibility to variola virus infection: Hu-PMC (with human peripheral blood mononuclear cells), Hu-NSG (with human haematopoietic stem cells) and Hu-BLT (implanted with bone marrow, liver and thymus fragments along with haematopoietic stem cells).). Hu-NSG and Hu-BLT were highly susceptible to infection, with a dose-dependent response and high mortality. They had practical advantages, including a reasonably defined incubation period of infection before onset of disease. Both animal models are demonstrated by the manufacturer to be stable over several months to years, providing the potential benefit to use them in the evaluation of next generation vaccine efficacy. The humanized mouse models did have disadvantages including high cost and lack of a robust number of lesions. The Hu-PMC mouse model had the additional disadvantage of a shortened period of time between displaying signs of disease due to variola virus infection and onset of graft versus host disease. Although the study had only been recently completed and not all analysis completed, the preliminary data suggest the humanized mouse models offer a good platform for research, for example on diagnostic assays and evaluation of treatments and vaccine efficacy.

9.3 In discussion, questions were raised about the practicality of the animal model and whether regulatory authorities would accept the results so obtained in considering licensure for use in humans. Comments were made about aspects such as controls, viral replication and the need for further work on pathology. Ongoing analysis of the study samples will address many of these comments. The good progress in the difficult area of animal models was noted.

10. Attenuated smallpox vaccine on the basis of LIVP vaccinia virus strain - Dr S. Shchelkunov

10.1 Dr Shchelkunov recalled that the Lister strain of vaccinia virus (LIVP), which had been used for human vaccination, causes some adverse reactions; BALB/c mice showed dose-dependent neurovirulence on immunization with the strain. Inactivation of some virulence genes does not affect the reproductive properties of the virus. The recombinant variant of vaccinia virus, 1421ABJCN, in which five genes encoding virulence factors have been inactivated, reproduced well in mammalian cells, and is less reactogenic and less neurovirulent than the LIVP strain. It showed the same immunogenicity as the parent virus and was equally protective against ectromelia virus. The 142ABJCN variant could be used as a new-generation, safe vaccine against both smallpox and other orthopoxvirus infections.

10.2 In terms of a time line for further research, preclinical work is expected to be completed in 2016. Depending on the outcome, plans for a project looking at neutralization of variola virus, a project with live virus, would be drawn up for submission to the Scientific Subcommittee for approval.

11. Use of live variola virus to evaluate antivirals against smallpox (monoclonal antibodies) - Dr V. Olson

11.1 Dr Olson noted that current considerations on preparedness in case of possible release of variola virus or re-emergence of smallpox indicated the need for two antiviral agents with different mechanisms of action to be licensed and available for use. Vaccinia immune globulin is licensed by the US Food and Drug Administration (FDA) for treatment of adverse events following smallpox vaccination, but it is in limited supply and is no longer mass produced. A few studies in the 1950s and 1960s had reported its potential value in conjunction with vaccination to prevent smallpox. As the Advisory Committee supports the development of new antiviral agents, work is focusing on compounds that target specific processes or functions in the virus but which are not essential for the human host.

11.2 In a collaborative project, a large series of monoclonal antibodies is being generated from the peripheral blood mononuclear cells of people vaccinated with vaccinia virus and survivors of smallpox and monkeypox and then tested for neutralizing ability against different orthopoxviruses. A mixture of six neutralizing antibodies was found to have strong binding properties against orthologous proteins for variola virus and vaccinia virus similarly. This activity was more efficient than that of vaccinia immune globulin. The mixture also protected mice challenged with a lethal dose of vaccinia virus; they survived and had low viral titres in the lungs. The next steps would be to test for protection against variola virus infection in vitro and in mice.

11.3 Dr Olson also reviewed compounds under investigation as antivariola virus agents, including tecovirimat, tyrosine kinase inhibitors, *Sarracenia purpurae* extract, brincidofovir, pyridopyrimidinones, and cellular kinase disrupters, all of which act at different stages of infection. Interest also is focusing on the role of CCR5 receptors, whose presence may lead to more efficient spread of some orthopoxviruses in infected hosts. Data on the growth of monkeypoxvirus and variola virus in different cell lines (but not vaccinia or cowpoxvirus), including the elimination of this advantage when the receptor was blocked, confirm this hypothesis. A medicine recently approved for treatment of HIV infection, maraviroc, functions by blocking entry of HIV through the CCR5 receptor, and future work could look at its role in the treatment of smallpox with a view to potentially repurposing the compound for this indication.

11.4 In response to questions about the time scale, Dr Olson said that work on in vitro evaluation of monoclonal antibodies against the intracellular mature virion of variola virus could take perhaps until March 2016. Work with live virus could not then start until the facility went live again after maintenance, and she could envisage in vitro work with live virus by 2017, if the results obtained justified further research. The Scientific Subcommittee did not give its approval for work with maraviroc.

12. Development of animal models to study the efficacy of therapeutic and preventive products against smallpox- Dr A. Agafonov

12.1 Dr Agafonov reported that outbred ICR mice at an early age (10-14 days old), which his group had previously been using, had limitations and outbred ICR mice (obtained from Vector's animal breeding facility) showed greater promise as models for smallpox research. They had similarities to humanized mice. The study involved the use of outbred 5-6-week-old ICR mice. They were infected intranasally with variola virus (Ind-3a strain). Viral replication in the lungs was noted at 4 and 5 days after inoculation and was seen in spleen macrophages at 3 days post-infection. The animal model held promise for evaluation of potential anti-variola virus compounds.

12.2 In response to questions in discussion on timeline, he estimated that further research could be completed within one to two years.

13. Use of live variola virus to support less-reactogenic vaccine development: continued evaluation of third-generation vaccines - Dr V. Olson

13.1 Dr Olson gave an update on the results of a collaboration with the National Institute of Infectious Diseases in Japan on evaluating LC16m8 vaccination regimens. The work aims to elucidate the neutralization capacity against variola virus of the humoral response after vaccination, work which is especially important for assessing third-generation vaccines such as Modified Vaccinia Ankara (MVA) which do not give the classical dermatological response (the "take"). Previous results had shown that LC16m8 vaccination induces significantly different neutralizing immune responses to two different strains of variola virus. In the recent work, sera were tested from 102 subjects vaccinated with LC16m8 and 24 vaccinated with Dryvax. Both vaccines elicited a similar neutralizing response against variola virus (strains VARV_BSH74_Sol and VARV_SLN68_258), but more people vaccinated with LC16m8 had 4-8-fold rises in neutralizing antibody than those vaccinated with Dryvax. Further work will determine the capacity of sera from subjects vaccinated with LC16m8 to neutralize extracellular enveloped virus and mature virus long after vaccination.

13.2 In addition, a non-inferiority clinical study (with 200 subjects) is planned with the vaccine manufacturer to compare IMVAMUNE® (an MVA-based vaccine, licensed in Europe and Canada for use against smallpox) and ACAM2000, with neutralization of variola virus as the secondary endpoint (see section 15 below).

13.3 For the neutralization work, the existence of the sera from vaccinees means that further tests would not take a long time.

14. Update on LC16m8 vaccine - Dr H. Yokote

14.1 Dr H. Yokote reminded the Advisory Committee that the live, attenuated LC16m8 vaccine is currently being stockpiled in Japan against a potential bioterrorism threat. The health ministry has recently reorganized its Smallpox Vaccine Research Group, whose aim is to produce a safer smallpox vaccine for routine vaccination. The members of the Group are the National Institute of Infectious Disease, the National Institute of Public Health and the pharmaceutical company Kaketsuken. Since 2014, the Group has published four scientific papers on the safety of the LC16m8 strain of vaccinia virus in mice and both clinical and animal studies with the vaccine. The studies showed that LC16m8 is about 5000 times safer than the Lister strain of vaccinia virus. Even in mice whose immunocompetence has been reduced by treatment with cyclosporine LC16m8 caused no clinical signs, unlike the Lister strain of vaccinia virus. Vaccination of immunodeficient mice also induced strong protective cell-mediated and humoral immunity, suggesting that the LC16m8 vaccine could be effective in immunodeficient people. A post-marketing surveillance study in 268 military personnel confirmed the vaccine's safety and efficacy, with no report of serious adverse events. Research will continue on neutralizing antibodies, seroconversion and the mechanism of LC16m8's efficacy with support from CDC.

15. Update on smallpox vaccine IMVANEX® (IMVAMUNE®) - Dr N. Samy

15.1 Dr N. Samy described the non-replicating smallpox vaccine, based on her company's strain of MVA virus, which has been licensed for use in the adult population as Imvanex® in Europe and approved for limited use against smallpox in Canada under the trade name Imvamune®. It is known as Imvamune® in the USA where it has been given pre-" emergency use authorization" status which signals that the FDA is ready to authorize it for use in certain populations during a declared public health emergency involving smallpox, and 28 million doses have been delivered to the US Strategic National Stockpile. It is administered subcutaneously in two doses (although one dose can induce long-term immune memory) and is produced in a liquid frozen formulation, which has a recommended shelf-life of two years at either -20 or -50 ˚C or five years at -80 ˚C.

15.2 The vaccine has been given to more than 7600 subjects, including people with atopic dermatitis and those infected with HIV. It has been shown to induce an equivalent protection to that of conventional smallpox vaccines in animal models, with a faster onset of protection (e.g. in terms of viral load in lungs and following exposure to ectromelia virus), and comparable immune responses in humans compared with that following Dryvax vaccination. Trials completed in 2015 confirmed the non-inferiority of a freeze-dried compared with the current liquid-frozen formulation as well as lot consistency and safety, in particular cardiac safety. It was safe and well-tolerated: most adverse events were typical of those seen with other injectable vaccines with no trends for unexpected and/or serious suspected adverse reactions due to the investigational product with no trends or clusters and no confirmed case of myo- or peri-carditis. Enrolment has begun for a Phase III clinical trial to demonstrate the non-inferiority of Imvamune® to the ACAM2000 vaccine (approved in the USA for active immunization against smallpox disease for persons determined to be at high risk for smallpox infection).

16. Progress towards approval and deployment of Arestvyr® (ST-246) - Dr D. Hruby

16.1 Dr D. Hruby updated the status of the development of tecovirimat (trade name Arestvyr®, formerly known as ST-246) for treatment of orthopoxvirus infections in adults. The product is stable up to 72 months; the possibility that the shelf-life can extend to 84 months is being studied. More than one million treatment courses have been delivered to the US Strategic National Stockpile to be used under the Emergency Use Authorization during a declared public health emergency involving smallpox, with a target of two million by 2018. CDC has developed an investigational new drug protocol for the investigational drug's emergency use in individual patients with serious complications following vaccination with ACAM2000.

16.2 Development of tecovirimat evolved in line with changes in the FDA's Animal Efficacy Rule for Developing and Testing Drugs and Biological Products when human efficacy studies are not ethical and field trials not feasible (the "Animal Rule") over the past decade.[8] In particular, it has responded to the requirement that the most conservative animal model will be used to estimate the dose reasonably expected to be efficacious in humans and the new requirement that the dose and regimen for humans should be selected to provide exposures that exceed those associated with the fully effective dose in animals, ideally by several-fold. The company developing tecovirimat used the non-human primate and rabbit models with monkeypoxvirus and variola virus, and is using rabbit/rabbitpoxvirus as a second surrogate model in order to satisfy the regulatory authorities.

[8] For guidance for industry released by FDA in October 2015, see http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm399217.pdf (accessed 24 February 2016).

16.3 Phase I and II trials have established the safety of tecovirimat. Preclinical trials began in 2015 on the use of oral capsules of tecovirimat for treatment of smallpox in adults (three 200 mg capsules twice a day for 14 days), a liquid formulation (e.g. for paediatric use), and an intravenous formulation (for those for whom oral administration is contraindicated). An alternative to oral capsules is being explored, in which the contents of a capsule are sprinkled on food or in drinks; FDA advised on this approach. No severe adverse event has been seen in clinical evaluations. Additional human safety studies are planned, and the company plans to begin clinical trials of its intravenous product in the first quarter of 2016.

16.4 Asked in discussion about the likely times scale of the additional trial, Dr Hruby said that he expected it to be completed within a year of its commencement, which was imminent. The FDA concurred with that projection, adding that they would expedite the review process. The development program for tecovirimat has benefited from the close collaboration between the company, FDA and the Biomedical Advanced Research and Development Agency (BARDA) of the US Department of Health and Human Services.

17. Update on the development of brincidofovir (CMX001) for smallpox - Dr R. Lanier

17.1 Dr R. Lanier outlined the status of the development of brincidofovir, a broad-spectrum inhibitor of DNA viruses including cytomegalovirus and variola virus. It has good oral bioavailability and pharmacokinetics, is available in tablet and liquid formulations, and has already been tested in more than 1000 patients against cytomegalovirus.

17.2 It is being tested for potential activity against variola virus in two animal models of smallpox with surrogate orthopoxviruses – rabbit/rabbitpoxvirus and mouse/ectromelia virus – in which the antiviral target of brincidofovir is the same as that in variola virus. Non-human primates were excluded because they were found to have a species-specific metabolism of the compound. In the rabbit tests, completed in 2015, brincidofovir showed significant protection when it was administered shortly after infection. No sign of resistance was evident in the rabbit model studies. A study of the efficacy of brincidofovir in the mouse/ectromelia virus model is scheduled to begin and be completed in 2016.

17.3 The work is being done in collaboration with BARDA, which provided a further grant of US$ 13 million in September 2015. Agreement was reached with FDA that both animal models are suitable for studying key aspects of smallpox. Potential advantages of brincidofovir for use in a public health emergency related to smallpox include oral administration, short course of therapy, low pill burden, compatibility with vaccination, favourable experience of use in special populations (including immunocompromised subjects) and lack of toxicity.

17.4 Asked in discussion about timelines, Dr Lanier said that the work on the rabbit/rabbitpoxvirus model was expected to be completed in 2016.

18. Food and Drug Administration: Perspective on the development and approval of smallpox medical countermeasures - Dr L. Borio

18.1 Dr L. Borio presented an overview of FDA's perspective on the development and approval of medical countermeasures against smallpox, which included: nucleic acid-based diagnostic assays for orthopoxvirus infections; investigational treatments (tecovirimat (Arestvyr®) and brincidofovir); and investigational vaccines (including ACAM2000 and Imvanex® – both in liquid-frozen and freeze-dried formulations). ACAM2000 and vaccinia immune globulin constituted part of the US Strategic National Stockpile, and both formulations of Imvanex® (liquid-frozen) were in advanced development.

18.2 For diagnostics, the main challenge is validation, and FDA is working closely with concerned parties and offering a flexible approach. The lesson has been learnt from the outbreak of Ebola virus disease in West Africa about the need for rapid and accurate tests at the point of care. The current smallpox diagnostic portfolio does not include development of rapid and accurate POC tests

18.3 For anti-smallpox drug development FDA relied on the Animal Rule and would accept non-variola orthopoxvirus models but with safety assessments in humans. If no benefit/risk assessment can be made on the basis of animal efficacy and clinical safety data, then properly-designed clinical trials in a smallpox event may be necessary. FDA finalized its guidance for developing products under the Animal Rule in 2015, which included the need for human doses to exceed those associated with efficacy in animals.

18.4 For vaccines, pre-emergency use authorization was in place for Imvanex®, and it is expected that the licensure of next-generation smallpox vaccine will be based on the traditional approval pathway. FDA recognized the continuing work on immunological assays, the need to consider the Animal Rule when the traditional approval pathway is not feasible, and the work on lot consistency and the comparison of liquid-frozen and freeze-dried formulations.

18.5 Participants welcomed the positive message. If animal efficacy and clinical safety data do not allow a benefit/risk assessment to evaluate whether products help or harm, properly-designed clinical trials in emergency settings may be required. Dr Borio said that this was another lesson from the Ebola virus disease outbreak. Such trials needed infrastructure and planning; the US Government was setting such planning in place, not only for smallpox but public health emergencies more broadly.

19. European Medicines Agency: progress on antivirals - Dr E. Pelfrene

19.1 Dr E. Pelfrene of the European Medicines Agency (EMA) outlined the different types of marketing authorization in the European Union, including procedures for exceptional circumstances, in which conventional drug development was not possible. For example, when comprehensive data were not available a set of full data was not likely to be delivered. Nevertheless the benefit/risk ratio needs to be positive based on assessment of the generated data (mainly derived from animal challenge studies and safety evaluation in healthy volunteers, using corresponding dosing [providing similar exposure kinetics]). Such exceptional authorizations were assessed annually and were valid for five years. The EMA's position corresponded to that of FDA, with an equivalent of the Animal Rule and similar general considerations for development and authorization of antiviral agents against smallpox. All applications for a new EU marketing authorization had to include a paediatric investigation plan, which may require the development of a separate paediatric formulation.

19.2 Comments were made in discussion appreciating the similarities in the approaches between FDA and the EMA.

19.3 The Advisory Committee noted the information, provided by the representatives of both the European Medicines Agency and the US Food and Drug Administration, that the development of protocols to test the effectiveness and safety of therapeutics in the field at the time of outbreaks was a prerequisite for the licensure of those agents.

20. Update on variola virus repositories biosafety inspection visits - Dr K. Kojima

20.1 Dr K. Kojima described the process of the biosafety inspection visits of the two repositories of variola virus undertaken by WHO in accordance with resolution WHA60.1. The latest set of visits had taken place in December 2014 for Vector and May 2015 for CDC; reports of both are in the final stages of publication. Since 2009, steady improvements had been recorded, with many issues addressed and closed. In 2014-2015 no immediate corrective actions had been identified and the number of findings had decreased compared with 2012.

20.2 The inspections have used the elements derived from the international laboratory biorisk management standard[9] to structure the inspections. However, a new rating system was introduced with a four-level scale: observations (reflecting positive statements about the institutions and their practices); priority 1 findings – improvement advisable; priority 2 findings – timely remedial measures required; and priority 3 findings – immediate corrective action required. One of the 16 elements on "good microbiological technique" could not be observed as the facilities were being

[9] European Committee for Standardization's Laboratory Biorisk Management Standard CWA 15793:2011.

decontaminated and not in use during the inspection period. Planning and preparation for the next set of inspection visits in 2016-2017 was underway.

21. General discussion: conclusion and recommendations

21.1 After an extensive general discussion, the Advisory Committee came to the conclusions and recommendations summarized below.

21.2 In particular, the Advisory Committee welcomed the report of the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology related to Smallpox and noted their overall conclusion that the nature of the risk of re-emergence of smallpox has changed significantly and is evolving.[10] The Advisory Committee discussed the key points regarding the implications for research and appreciated the need for enhancing preparedness measures to deal with the potential consequences of the synthesis and possible re-emergence of variola virus. The points below are a result of the discussions and conclusions on the issues raised in the report.

21.3 Future research needs and preparedness activities

21.3.1 The Advisory Committee considered the possibility of increasing the number of research sites allowed to work with live variola virus but was strongly of the view that there was no need for increasing the number of such research sites. Based on a risk-benefit assessment, the Advisory Committee was of the opinion that the risk associated with any increase in the number of research sites beyond the existing two authorized repositories outweighed the benefits.

21.3.2 Recognizing the need for increased preparedness measures to respond to the potential synthesis and possible re-emergence of variola virus, the Advisory Committee encouraged the expansion of expertise in the area of laboratory biosafety and biosecurity and diagnostics for this purpose.

21.3.3 The Advisory Committee identified, as a clear priority, the need for more laboratories with diagnostic capacity but without them holding or using live variola virus. It recommended that enhancing the capabilities for differential diagnosis of smallpox from other similar diseases should include building national and subnational capacities for detection of other poxviruses, such as monkeypox, in countries that are endemic for those diseases.

[10] the report of the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology related to Smallpox, document WHO/HSE/PED/2015.1 (http://apps.who.int/iris/bitstream/10665/198357/1/WHO_HSE_PED_2015.1_eng.pdf, accessed 13 January 2015).

21.3.4 The Advisory Committee also recommended that point-of-care diagnostic tests for variola virus infection be developed and that their use should be coordinated to assure accuracy of testing, especially in low disease prevalence settings, in order to help various national, regional and global laboratory networks to improve their smallpox diagnostic capabilities. The WHO smallpox laboratory technical working group has developed a framework to support regional test capacity.

21.3.5 As a way to increase national preparedness capacities, the Advisory Committee also recommended to increase the training of additional staff to:

- be able to work with dangerous pathogens, noting that this work did not necessarily require the use of live variola virus, as the emphasis was on diagnosis rather than research; and
- recognize smallpox cases, noting that CDC had produced an excellent flowchart of the algorithm for this process some time ago.

21.4 Review of the terms of reference, research agenda and composition of the Advisory Committee

21.4.1 Given the change in risk of emergence of smallpox due to synthetic biology technology, the Advisory Committee reviewed its terms of reference (see Annex 4) and concluded that they were broad enough to include the area of synthetic biology technology if needed. In light of the public health implications identified by the Independent Advisory Group in its report on synthetic biology, the Advisory Committee recognized the need to consider the potential for re-emergence of smallpox as part of its remit.

21.4.2 The Advisory Committee gave special attention in its review of the current research agenda to assess whether there are or will be additional needs for smallpox control measures in case of re-emergence of a synthesized and/or modified variola virus. There was general agreement that the areas of current research – focusing on translational outcomes for diagnostics, therapeutics and vaccines – remain important.

21.4.3 In order to help the Advisory Committee foster greater preparedness to deal with the implications of synthetic biology, the members agreed that additional members with appropriate expertise related to new technologies, such as synthetic biology, would be welcome.

21.5 Revision of the WHO Recommendations on the distribution, handling and synthesis of variola virus DNA and its dissemination

21.5.1 With regard to the point raised by the Independent Advisory Group on adapting the rules and regulations concerning access to and use of variola virus DNA, the Advisory Committee revised the

WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA (see Annex 5). The Advisory Committee emphasized that most of the original recommendations had not changed but have been expanded to include: explanatory text about their rationale; clarification of the position on compliance with the guidelines; and further clarification that the limit of 500 base pairs for synthesis of DNA fragments applies to diagnostics. The Advisory Committee also included a definition of the term "variola virus DNA" for the purpose of this guidance. The Advisory Committee agreed that, if researchers want to express one or more variola virus genes to produce variola virus protein, they need to seek permission from the WHO Secretariat. The process would pass through the Chairman of the Advisory Committee's Scientific Subcommittee.

21.5.2 The Advisory Committee stated that it was highly desirable for the WHO Secretariat to develop, as a matter of urgency, an online application form that would facilitate accelerating the processing of such requests for variola virus DNA.

21.5.3 The Advisory Committee strongly recommended that the revised recommendations on the distribution, handling and synthesis of variola virus DNA be widely distributed and adopted by Member States as part of their national biosafety and biosecurity regulations. This action would ensure that the recommendations became mandatory and enforceable by each nation that adopted them and therefore harmonized between Member States.

21.5.4 Other mechanisms were needed to further disseminate the recommendations widely, including their publication in journals, adoption by associations, and implementation by funding agencies.

ANNEX 1

Abstracts of presentations

RESEARCH PROPOSALS SUBMITTED TO WHO IN 2014-2015: DECISION AND COMMENTS OF THE SCIENTIFIC SUBCOMMITTEE

Project proposals from CDC

- Animal model using humanized mice
  - Approved, regulatory acceptance will be challenging
- Protein-based diagnostic assays
  - Approved
- Antivirals
  - Denied
  - The reviewers were concerned about the limited progress with previously described compounds. Prioritization and a lack of data (using other viruses) was also highlighted as concerns
- Variola-derived materials for diagnostics
  - Approved, but numbers needed to be stated explicitly, "sequence and destroy" was noted as a positive strategy
- Vaccine/virus specificity
  - Approved, although some doubts were expressed about the reliability of the historical data cited to justify the project

Revised proposals from CDC

- Prophylactic monoclonal antibodies
  - Approved.
- Kinase inhibitors
  - Denied.
  - Although scientifically interesting, the research was not seen as meeting the "essential" standard.
- Maraviroc
  - Denied.
  - Although viewed as possibly scientifically interesting, it also not viewed as "essential" research. The lack of animal models would hazard regulatory approvals.

Project proposals from Vector

- Antivirals
  - Revisions were requested
  - Concerns were expressed about the slow rate of demonstrated progress, and a lack of clarity regarding which compounds would be tested. Whether it would be essential to use variola virus for these studies was also questioned.
- Animal models
  - Revisions were requested
  - Concerns were expressed about the slow rate of demonstrated progress and many reviewers expressed scepticism about the utility of the proposed model (nude mice).
- Neutralizing activity in sera
  - Approved, but Vector was requested to incorporate time limits on the proposed research.

Revised proposals from Vector

- Animal models

  - An immunocompetent (ICR/CD-1) model was introduced, and justified based upon preliminary data presented to the Advisory Committee on Variola Virus research at its sixteenth meeting (in 2014)
  - Some time-limited further experimentation was approved
- Antivirals
  - More details were provided including citations. This included IC_{50} and selectivity index (SI) data suggesting possible utility.
  - Some limited further experimentation was approved.

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

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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 the Federal State Research Institution 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 the WHO African Region, the WHO Region of the Americas (South America), and the WHO South-East Asia, European and Eastern Mediterranean regions.

The variola virus strains are stored in the repository in a lyophilized or frozen form as well as in the form of primary material samples from patients (scabs).

In 2015, the Ind-3a and Butler variola virus strains were grown in Vero cells and then used in the smallpox research projects approved by WHO:

1. *Discovery of new antivirals for smallpox treatment and prevention*

2. *Assessment of the neutralizing activity of vaccinee blood sera using live variola virus*

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

Research towards the above-stated research goals using live variola virus is going to be continued in 2016–2017.

THE WHO COLLABORATING CENTER FOR SMALLPOX AND OTHER POXVIRUSES AT THE CENTERS FOR DISEASE CONTROL AND PREVENTION ATLANTA, GA: 2015 REPORT ON THE VARIOLA COLLECTION

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The WHO Collaborating Center for Poxviruses in Atlanta, Georgia, United States of America, continues to maintain one of two consolidated, international collections of variola virus strains. The laboratory space was in active use from October 2014 through late March 2015; the laboratory underwent decontamination prior to preventative maintenance in April 2015. The WHO inspection team visited the Collaborating Center at CDC for 10 days during late May 2015. The laboratory became operational again in late July 2015. No new variola virus seed pools were added to the inventory between 2014 and 2015. A working stock of crude variola virus (strain VARV_BSH_74_sol) has been prepared for use in evaluating protein-based diagnostics. The six vials of variola virus found on the National Institutes of Health Bethesda campus in July 2014 have been destroyed, as witnessed by WHO representatives in February 2015. WHO-approved research activities which have utilized variola virus from the inventory within the last year have focused on: determination of whether humanized mice are viable modes of smallpox disease, neutralization potential of human monoclonal antibodies, optimization of protein based diagnostic assays, and evaluation of sera from vaccination regimens to evaluate efficacy based on variola virus neutralization. In May 2013, variola virus samples were removed from the repository for WHO-sanctioned protocols. These original scabs or homogenates were processed (not propagated) for sequencing under the WHO approved protocol. To date, 135 original samples have been processed; 42 samples have complete genomic sequence. The preliminary analysis shows that the sequencing coverage has considerable variation from sample to sample, we are developing variola virus DNA enrichment protocol to improve the Next Generation Sequencing results for the clinical samples.

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

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The ability to validate nucleic acid-based and protein-based diagnostic capacity is critical for early detection and recognition of smallpox should a bioterror event result in reintroduction. The consequences of either false negatives or false positives will significantly impact our global public health system.

DNA diagnostics

In 2010, several strains of cowpoxvirus showed cross-reactivity to a previously-validated variola-specific signature. Identification and characterization of unique variola virus signature sequences have been assessed for specificity and sensitivity to variola virus. Data from a multi-centre study (using a plasmid containing non-contiguous target fragments (<500 base pairs each)) were presented to the United States Laboratory Response Network (LRN) Technical Review Committee for approval prior to deployment to approved LRN laboratories. The LRN has been working with CDC in preparing a submission of the diagnostic assays for FDA approval, which is nearing completion.

Protein diagnostics

Since late 2011, studies have also been continuing on monoclonal antibody characterization and viral antigen capture assays. In the past year, work has focused on standardizing virus preparations, re-subcloning promising hybridomas, and working on bioinformatics-guided approaches to variola-specific monoclonal antibody design. Several peptide-derived monoclonals have been screened for reactivity against gamma-irradiated and live variola virus samples and specificity determined in comparison with other orthopoxviruses. Several promising monoclonal antibodies have been identified. New diagnostic assays utilizing these antibodies may allow for rapid detection and speciation of variola virus within antigen-capture assays. Another aspect of protein diagnostics involved generation of variola virus proteome microarray chips. The chips will be utilized for profiling antibody responses against individual proteins to compare and contrast immune-dominant proteins followed by vaccination or infection. It has been used successfully to identify protein targets against variola virus reactive monoclonal antibodies.

DISCOVERY OF NEW ANTIVIRALS FOR SMALLPOX TREATMENT AND PREVENTION

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

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The discovery of novel antiviral agents for treatment and prevention of smallpox remains important. In 2014, the State Research Centre of Virology and Biotechnology Vector tested about 120 new chemically synthesized compounds of the LPP, FAS-V, KU and Sag series against surrogate orthopoxviruses (vaccinia virus, cowpoxvirus, and ectromelia virus). For the purpose of further research, 10 compounds were selected that showed greater antiviral effects, namely four compounds of the LPP series, one compound of the FAS-V series, and two compounds of the KU series. During 2015, the antiviral effects of these chemical compounds against the Ind-3a variola virus strain were studied in vitro.

One compound, a derivative of 3-amino-5-nitropyridine, was found to have a relatively high anti-orthopoxviral activity, with a selectivity index >72. Among the 10 compounds examined during this stage of work, representing chemical classes of compounds other than tecovirimat (ST-246) or NIOCH-14, no highly potent compound against variola virus in vitro was discovered.

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

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

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Neutralization of variola virus (VARV) is of great importance for the evaluation of the specific activity of vaccines that do not cause typical pox signs that form as a result of using conventional cutaneous vaccines.[11] So, the most reliable data, allowing judgments to be made about the effectiveness of protective humoral immunity formation while looking into finding an optimal application scheme of such vaccines, across various regimens and methods of vaccination, are the neutralizing activity values of antibodies in the sera of vaccinees, using variola virus. Previously, the State Research Center of Virology and Biotechnology Vector conducted variola virus neutralizing activity studies of the sera of human volunteers vaccinated with a conventional cutaneous smallpox vaccine as well as those immunized with a tablet formulation of the investigational recombinant live vaccine, Revax-BT, for oral administration.

A question needing response was how long the humoral immune response to VARV lasted in the volunteers immunized with the smallpox vaccines, and what were the levels of antibody generation in another animal species (rabbits) commonly used at the stage of pre-clinical vaccine studies in response to oral administration of a microencapsulated recombinant cell-based vaccine or genetically modified cell-based vaccine.

For experiments, the Ind-3a variola virus strain from the State Collection of Viruses and Rickettsiae maintained at Vector was used, and we tested serum samples from Vector employees collected 1 month and 18-36 months after a two-step immunization with inactivated and cutaneous conventional live vaccines. In addition, rabbit serum samples were used that were collected 30 days after oral immunization with a microencapsulated recombinant cell-based vaccine. The antibody levels in the sera were determined by neutralization. Conventional approaches of statistical analysis of data obtained were used.

The study of the variola virus neutralizing activity of sera from individuals vaccinated via a two-step primary immunization with the inactivated and conventional live cutaneous vaccines showed continued high levels of antibodies that neutralized the Ind-3a strain of variola virus, even 1.5 to 3 years after immunization. The study of the neutralizing ability of sera, collected 1 month post-immunization of rabbits (chinchilla) with the investigational cell-based microencapsulated recombinant vaccine, revealed they had neutralizing antibodies against the Ind-3a strain of variola virus.

Thus, the primary vaccination of individuals with the inactivated and conventional live cutaneous vaccines[12] results in the formation in their sera of antibodies neutralizing the Ind-3a strain of variola virus, and the levels of these antibodies remain high for, at least, three years. The oral immunization of rabbits (chinchilla) with the cell-based microencapsulated recombinant vaccine induces the formation in their sera of antibodies neutralizing the Ind-3a strain of variola virus.

[11] Report of the thirteenth meeting of the Advisory Committee on Variola Virus Research (Geneva, 31 October – 1 November 2011), document WHO/HSE/GAR/BDP/2011.2 (http://www.who.int/csr/resources/publications/WHO_HSE_GAR_BDP_2011_2/en/, accessed 17 January 2016).

[12] According to Russian Federation Guidance MU 3.3.1.2044-06 and MU 3.3.1.2045-06.

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

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The discovery of a novel, permissive/representative animal model system would facilitate the development of next-generation, safer smallpox vaccines and therapeutics. Humanized mice have become an invaluable tool for modelling human biology and disease as they provide an excellent alternative to non-human primates. Researchers are using them to investigate human-specific disease pathogenesis of multiple viruses. The potential utility of a rodent challenge model using variola virus – supplied from inbred populations with minimal intrinsic variability, greater availability of specific immunologic reagents, and ease of animal handling – makes it of great interest to determine if they are susceptible to disease.

We selected three different types of humanized mice (PBMC [hu-PBMC NSG (human peripheral blood mononuclear cells (PBMCs) engrafted into NSG (NOD mutation, SCID mutation, interleukin 2 receptor gamma chain mutation) mice], NSG [hu-CD34 NSG (haematopoietic stem cells (HSCs) injected into NSG mice)], and BLT [hu-BLT NSG (bone marrow, liver, and thymus fragments implanted into NSG mice also receiving HSCs)]) from Jackson Laboratories (Bar Harbor, Maine) to evaluate for susceptibility to variola virus challenge. We intranasally challenged all three different types of humanized female mice [15 weeks old (BLT and NSG) or 8 weeks old (PBMC)] with a range of doses of variola virus [$5x10^5$ or $5x10^7$ plaque forming units (pfu)]. Mice were co-housed in groups of 2-4 animals per ventilated cage in the High Containment Laboratory (BSL-4) and all mouse handling was conducted on a down-draft table. Control mice were challenged with PBS or gamma-irradiated variola virus using identical inoculation methods. Standard mouse husbandry practices with modifications to accommodate the increased sterile environment were performed during the experiment in accordance with CDC Institutional Animal Care and Use Committee (IACUC) guidelines under approved protocol 2671. Daily observations of the animal's food consumption, activity level, and general appearance were recorded. Clinical criteria were used to assess animals for euthanasia criteria. Three times a week body weights were recorded, complete skin exams were undertaken, and oral swabs were collected while the animals were under anaesthesia with 3%-5% isoflurane gas. At day 21 post infection, all surviving animals were humanely euthanized for necropsy. Clinical signs of disease were more pronounced in two of the humanized mouse strains (BLT and NSG) that were challenged with live variola virus, in a dose-dependent manner. High mortality was seen in both the NSG [87.5%] and BLT [100%] mice (both the 5×10^5 to 5×10^7 pfu challenge groups) and additionally the animals challenged with the high dose of variola virus (4 per mouse strain) succumbed to disease earlier (day 13-17 post infection) than those challenged with the low dose (day 13-21 post infection). Furthermore, both the BLT and NSG mice displayed an incubation time of at least 13 days post infection before succumbing to disease. The third mouse strain (PBMC) did not display similar sensitivity to variola virus challenge until later in the study (only 1/8 of the PBMC mice had to be euthanized on day 19 post infection based on the pain score). Clinical signs observed included decreased activity/unresponsiveness to stimuli, ruffled hair, very few cutaneous lesions (swabs of lesions failed to yield positive viral DNA signals), and weight loss. Gross necropsy findings of animals that were found dead or euthanized were primarily observed in the liver; multifocal hepatic lesions were observed in 4 BLT mice and 2 NSG mice. Despite clinical signs and mortality in the BLT and NSG mice, there was minimal shedding of viable virus in oral secretions (4 oral swab samples were positive for viral DNA but only two contained low levels of viable virus (<250 pfu/swab)). However, analysis of liver samples from a subset of challenged animals demonstrated high loads of viable virus (up to 10^8 pfu/g tissue) while those challenged with gamma-irradiated virus did not. Serum is being tested to determine seroconversion. Both the BLT and NSG mice have the potential to function as in vivo models of smallpox disease for testing of efficacy for therapeutics and vaccines. Unfortunately, the PBMC mice did not display signs of disease until much later (19 days

post infection) which nears the time when they develop graft versus host disease (4-5 weeks), limiting the usefulness of these animals as a model system.

ATTENUATED SMALLPOX VACCINE BASED ON LIVP VACCINIA VIRUS STRAIN

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Based on the LIVP strain of vaccinia virus (VACV) that is used for human vaccination, a recombinant variant 1421ABJCN was created by disruption of five genes encoding such virulence factors as haemagglutinin (*A56R*), gamma-interferon-binding protein (*B8R*), thymidine kinase (*J2R*), complement-binding protein (*C3L*) and Bcl2-like inhibitor of apoptosis (*N1L*). It has been shown that inactivation of selected virulence genes does not affect reproductive properties of VACV in cultures of mammalian cells. The 1421ABJCN strain is characterized by a significantly lower reactogenicity and neurovirulence compared to the original LIVP strain. During the intracerebral administration, the production of 1421ABJCN virus in the brain of newborn mice is reduced by three orders of magnitude compared with the original VACV LIVP strain. It has been shown that the recombinant 1421ABJCN variant, when injected subcutaneously into mice, induces neutralizing antibodies on a par with the original LIVP strain VACV, and provides mice complete protection against doses of ectromelia virus that would be highly pathogenic. The recombinant variant of VACV can be used as a new-generation, safe, live vaccine for the prevention of smallpox and other orthopoxvirus infections.

USE OF LIVE VARIOLA VIRUS TO EVALUATE ANTIVIRAL AGENTS AGAINST SMALLPOX

Dr V. Olson

Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

The primary objective of smallpox bioterrorism preparedness is to save lives if smallpox somehow re-emerges. 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. Vaccinia immune globulin (VIG) is licensed by the Food and Drug Administration for treatment of complications from vaccination with vaccinia virus. However, VIG is in limited supply since it is no longer mass produced after the eradication of smallpox. There is also some historic literature that suggests that treatment with immune products from persons infected with variola virus may provide more protection against smallpox.

ACVVR has continued to support the evaluation of new compounds, given the uncertainties of the drug development process. This project focuses 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.

Research at the Vanderbilt University Medical Center (Nashville, Tennessee, USA) has utilized a highly-optimized human hybridoma technology to create a large panel of monoclonal antibodies (mAbs) from vaccinia virus immunized persons or persons who survived either monkeypoxvirus or variola virus infection. These mAbs were directed against multiple viral proteins, with D8 being the most prevalent. Further characterization of the mAbs identified several that are capable of complement-dependent neutralization of orthopoxvirus. Most intriguingly, when several mAbs were used in combination, they were found to provide complete protection of mice against a lethal respiratory challenge with vaccinia virus. The ability of these mAbs to neutralize variola virus in vitro using our classic plaque reduction neutralization assay as well as their ability to limit virus spread will be determined. These mAbs may provide added therapeutic benefit over the current VIG, which is in limited supply.

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

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

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We have previously demonstrated the possibility of using outbred ICR mice at an early age (10-14 days old) as a laboratory model to evaluate the efficacy of anti-smallpox drugs.[13] However, the use of this animal model has some limitations owing to their imperfect (developing) immune system and reproduction of only the first component of the infectious process (variola virus propagation only in the respiratory organs of mice). However, outbred ICR mice have a distinct advantage over inbred animal species, first because of their lower cost and, secondly, because they reflect the diversity of the human population, which is essentially outbred (inter-family, inter-ethnic and interracial cross-breeding). Previously in some experiments we used, as a control, adult outbred (immunocompetent) ICR mice in conducting studies involving young outbred ICR mice (10-14 days old) to develop a laboratory model to assess the efficacy of anti-smallpox drugs. The results of the studies, conducted with the use of such control animals, showed promise towards development, on their basis, of a laboratory model to evaluate the efficacy of the anti-smallpox drugs.

The study involved the use of outbred 5--6-week-old ICR mice obtained from Vector's animal breeding facility and the Ind-3a strain of variola virus from the State Collection of Viruses and Rickettsiae maintained at Vector. The mice were infected by the intranasal route. The primary monolayer culture of macrophages in ICR mice was derived from a homogenate prepared from their spleen.

During variola virus infection of the 5--6-week-old (immunocompetent) ICR mice at selected variola virus doses (4.5 and 5.5 lg PFU), no visible clinical symptoms of disease were observed. At the same time, 4 and 5 days following the intranasal infection of these animals at the dose of 4.5 lg PFU variola virus, reliable reproduction of the virus in the lungs was observed. During an electron microscopy examination of the macrophage monolayer of the spleen of outbred adult (immune) ICR mice 3 days after variola virus infection at the dose of 0.017 PFU/cell, virus reproduction in the spleen macrophages was noted.

Thus, it has been demonstrated that outbred 5--6-week-old (immunocompetent) ICR mice can be infected via respiratory introduction of variola virus, as reflected by reliable accumulation of the virus in the lungs 4 and 5 days post-infection, and can propagate the pathogen in the macrophages of their spleen. These facts, supporting the propagation of the virus in the body of 5--6-week-old outbred (immunocompetent) ICR mice, indicate the prospects for their development as a laboratory model for evaluation of the efficacy of the anti-smallpox drugs, using our previously applied approach, which is focused on the study of the infection process.

[13] Titova KA, Sergeev AA, Zamedyanskaya AS et al. Using the ICR and SCID mice as animal models for smallpox to assess antiviral drug efficacy. J. Gen.Virol., 2015; doi: 10.1099 / vir.0.000216.

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

Dr V. Olson

Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

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 mature virion (MV) and extracellular enveloped (EEV) forms of variola virus will provide a measure of efficacy. The role of variola virus neutralization as a marker for vaccine efficacy is particularly valuable for the evaluation of vaccines that do not elicit a "take", the traditional measure of vaccine success.

This presentation updated results from a collaboration with Japanese government and industry scientists to evaluate LC16m8 vaccination regimens for variola virus MV neutralization. Sera from 67 LC16m8 vaccinees and 19 Dryvax vaccinees were evaluated for neutralization ability against variola virus. Vaccination with LC16m8 induced neutralizing immune response that was not significantly different than vaccination with Dryvax (P >0.1). Overall, LC16m8 vaccination appears to result in similar neutralizing immune response compared to Dryvax vaccination. When rises in titre were compared, we saw similar neutralization against VARV_BSH74_sol regardless of which vaccine was used (LC16m8 or Dryvax). However, there was a higher percentage of LC16m8 vaccinees capable of obtaining a 4-fold or 8-fold rise in neutralization titre against VARV_SLN68_258 compared to Dryvax vaccinees. Unfortunately, owing to limited quantities, we were unable to determine the ability of the same sera to neutralize the two different strains of variola virus. Finally, we have also initiated discussions on how to determine neutralizing capacity of MVA vaccinee sera against variola virus. Initial steps will provide data on the dynamic range and reproducibility of our traditional plaque reduction neutralization assay. These data will be essential for future submissions towards regulatory approval for these "third-generation" vaccines.

UPDATE ON A THIRD-GENERATION SMALLPOX VACCINE LC16m8

H. Yokote

Business Development Department, Vaccine Division, The Chemo-Sero-Therapeutic Research Institute (Kaketsuken), Kumamoto, Japan

In the 1970s, an attenuated replication-competent vaccinia virus, the LC16m8 strain, was developed from the Lister strain by serial passaging in primary rabbit kidney cells at a low temperature, during efforts to establish a safer strain.

Currently, the LC16m8 vaccine is intended for an emergency use. Kaketsuken has well-established manufacturing processes for the LC16m8 vaccine and is able to supply a certain amount of the vaccine with high quality in a short time, as necessary.

The Ministry of Health, Labour and Welfare in Japan recently reorganized the Smallpox Virus Research Group to evaluate safety, efficacy, characterization and quality of the LC16m8 vaccine.

Four reports have been published on the LC16m8 vaccine with the results of studies of safety evaluation in animals and efficacy evaluation in immunocompromised animals conducted by the members of the Smallpox Virus Research Group as well as a post-marketing surveillance in healthy adults conducted in collaboration with the Smallpox Virus Research Group.

UPDATE ON THE NON-REPLICATING SMALLPOX VACCINE IMVANEX® (IMVAMUNE®)

N. Samy

Clinical Development, Bavarian Nordic, Martinsried, Germany

Modified Vaccinia Ankara (MVA-BN®; IMVAMUNE® in Canada and the United States of America) is a live, highly attenuated, viral vaccine under advanced development as a non-replicating smallpox vaccine. The Government of the United States of America added IMVAMUNE® to its Strategic National Stockpile under a pre-emergency use authorization to be potentially used in all human immunodeficiency virus-infected and atopic dermatitis subjects following a declared smallpox emergency. In 2013, MVA-BN® was licensed in the European Union under the trade name IMVANEX® and was added to the WHO stockpile as a non-replicating smallpox vaccine. Since then, Bavarian Nordic has more than doubled the clinical database for IMVANEX® by recently completing two additional clinical trials. A placebo-controlled Phase III trial (4000 subjects, of whom 3000 received IMVANEX®) demonstrated equivalence of three consecutively produced vaccine lots in terms of neutralization antibody titres and confirmed the excellent safety profile of IMVANEX® in a large study population. A Phase II non-inferiority trial comparing the immunogenicity and safety of two formulations of IMVANEX® (650 subjects) demonstrated that antibody responses induced by the freeze-dried formulation were non-inferior to those induced by the liquid frozen formulation when measured by vaccinia-specific ELISA and plaque reduction neutralization assay (PRNT). In both clinical trials robust humoral immune responses were observed, with PRNT geometric mean titres of 82-117 and seroconversion rates of 99%-100% across all groups. In addition, strong cellular immune responses were induced by both formulations of IMVANEX®.

A randomized open-label Phase III non-inferiority trial is currently ongoing with aim of directly comparing indicators of efficacy of IMVANEX® to the replicating smallpox vaccine ACAM2000® (220 subjects per group). Efficacy of IMVANEX® will be demonstrated by assessing non-inferiority to ACAM2000® in terms of vaccinia-specific PRNT antibody response and by showing that vaccination with IMVANEX® prior to administration of ACAM2000® results in an attenuation of the "take", indicating that IMVANEX® induces neutralizing antibody levels sufficient to prevent viral replication.

After vaccinating more than 7600 subjects with IMVANEX® in completed and ongoing clinical trials, no case of myocarditis, confirmed pericarditis, endocarditis or any other type of cardiac inflammatory disease (or related syndromes) was reported, indicating that IMVANEX® does not cause any inflammatory cardiac events as observed with conventional, replicating smallpox vaccines like Dryvax® and ACAM2000®.

In conclusion, results of the recently completed clinical trials have confirmed the strong immunogenicity and safety profile of IMVANEX®, suggesting this non-replicating vaccine as the ideal candidate for being included in pre- and post-event smallpox preparedness plans, in particular for protecting first-line responders, military personnel and populations with increased rates of severe side effects from replicating vaccines, such as immunocompromised and eczema patients.

TPOXX (TECOVIRIMAT): UPDATE ON DEVELOPMENT AND DELIVERY

D.E. Hruby

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TPOXX (tecovirimat) is an antiviral being developed for the treatment of symptomatic orthopoxvirus diseases: smallpox (variola), monkeypox, cowpox and vaccinia. Several formulations (oral, intravenous and sprinkle) are being provided to provide protection to the entire populace. A robust manufacturing chain is in place and finished product is actively being delivered to the US Strategic National Stockpile. Current development efforts are centred on satisfying recent regulatory guidance that dictates triangulation of efficacy and dosing parameters between two animal species and the human. As extensive non-human primate studies have already been completed, efforts have focused on design and execution of a group of rabbit/rabbitpoxvirus studies and human clinical studies at the probable human dose. The results obtained appear to support the concept that non-human primates are the most conservative species and as such should allow finalization of the human dose in consultation with the appropriate regulatory agencies.

UPDATE ON THE DEVELOPMENT OF BRINCIDOFOVIR FOR SMALLPOX[14]

E.R. Lanier

Chimerix Inc., Durham, North Carolina, United States of America

Brincidofovir (BCV) is a potent in vitro inhibitor of multiple dsDNA viruses including adenovirus, cytomegalovirus and variola virus (VARV). Following intracellular metabolism of BCV to cidofovir diphosphate (CDV-PP), CDV is incorporated by the viral polymerase into nascent chain virus DNA, which subsequently inhibits DNA replication. Key attributes of BCV compared with cidofovir include increased in vitro potency, decreased nephrotoxicity, and ease of use, as BCV is orally bioavailable. Both compounds have favourable resistance profiles. BCV is available in tablet and liquid formulations and manufacturing has been validated at commercial scale. It has been administered to more than 1000 human subjects, including patients with renal and/or hepatic impairment, and paediatric subjects as young as one month of age. The US Food and Drug Administration has agreed that the intradermal rabbitpox model and the intranasal mouse ectromelia virus model are acceptable animal models to support development of BCV for treatment of smallpox. In 2015, a randomized, blinded, placebo-controlled study of the efficacy of BCV treatment in New Zealand white rabbits infected with rabbitpoxvirus was completed. At the time of confirmed fever, infected rabbits were randomized to 1 of 5 blinded treatment groups: placebo, immediate BCV, or BCV delayed by 24, 48, or 72 h. Animals received either placebo or a BCV regimen consisting of an initial 20 mg/kg oral dose, followed by two 5 mg/kg doses at 48-h intervals; doses were scaled from exposures in humans. A significant reduction in mortality was observed when BCV was initiated immediately, 24, and 48 h after the onset of fever (P <0.05 vs placebo, Fisher's exact test). A reduction in mortality was also observed when BCV was initiated 72 h after onset of fever, but this was not significant (P = 0.091 vs placebo). Further, BCV treatment was associated with reduced mean peak viral load compared with placebo during the course of the disease, as well as reductions in the number of plaque-positive samples and in mean PFU/mL of positive samples (P <0.05 vs placebo). A randomized, blinded, placebo-controlled study of the efficacy of BCV in the ectromelia virus model is scheduled to begin in 2016.

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

FDA PERSPECTIVE ON THE DEVELOPMENT AND APPROVAL OF SMALLPOX MEDICAL COUNTERMEASURES

Dr L. Borio

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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 approval[15]—as well facilitating timely access to MCMs in the event of a public health emergency through an appropriate regulatory mechanism.

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 2014 ACVVR meeting as well as some of the continuing challenges.

Smallpox MCMs present unique and complex regulatory challenges because the scientific evidence upon which regulatory decisions are based is challenging to obtain owing to the fact that there is no smallpox disease in the world and animal models that adequately represent smallpox disease are not available.

Working closely with smallpox MCM developers, FDA has established feasible and appropriate regulatory pathways for their approval. The focus of FDA's interactions with MCM developers is on providing feedback on proposed studies to support clinical safety, pharmacokinetic, and animal model efficacy studies for antiviral drugs, pivotal efficacy studies and bridging studies for the attenuated vaccines, and requirements for the validation of diagnostic tests. In 2015, FDA issued final guidance on developing products under the Animal Rule. The guidance enhances and clarifies FDA's expectations and recommendations on developing products under the Animal Rule based on comments received to previous draft guidance and the aggregate experience to date in developing products under the Animal Rule.

[15] 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.

ANNEX 2

Agenda of the seventeenth meeting of the Advisory Committee on Variola Virus Research

12 and 13 January 2016

Salle D, WHO headquarters

Geneva, Switzerland

DAY ONE – 12 January 2016

9:00 - 9:15	OPENING – **Dr Bruce Aylward**, Executive Director ad interim Outbreaks and Health Emergencies and Special Representative of the Director-General for the Ebola Response
	Election of Chair

VARIOLA VIRUS REPORTS

9:15 – 9:45	Report of the secretariat - WHO secretariat – **A. Costa**
9:45 – 9:55	Report of the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology related to Smallpox – **M. Sprenger**
9:55 – 10:10	Update on research proposals submitted to WHO in 2014-2015 – **D. Evans**
10:10 – 10:20	Report on the variola virus collection at the WHO Collaborating Center Repository in VECTOR, Koltsovo, Novosibirsk, Russian Federation – **A. Agafonov**
10:20 – 10:30	Report on the variola virus collection at the WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America – **I. Damon**
10:30 – 11:00	**Tea/coffee break**

VARIOLA VIRUS RESEARCH UPDATE – 2014-2015

11:00 – 11:20	Use of live variola virus to maintain – **I. Damon**
11:20 – 11:40	Discovery of new antivirals for smallpox treatment and prevention/ Assessment of the neutralizing activity of vaccinated human volunteers blood sera and those of immunized laboratory animals using live variola virus – **S. Shchelkunov**
11:40 – 12:00	Use of live variola virus to determine whether mice are a suitable animal model for human smallpox – **V. Olson**
12:00 – 12:20	Attenuated smallpox vaccine on the basis of LIVP vaccinia virus strain – **S. Shchelkunov**
12:20 – 12:30	Use of live variola virus to evaluate antiviral agents against smallpox – **V. Olson**
12:30 – 12:40	Development of animal models to study the efficacy of therapeutic and preventive products against smallpox – **A. Agafonov**
12:40 – 13:00	Use of live variola virus to support less-reactogenic vaccine development: continued

	evaluation of "third" generation vaccines – **V. Olson**
13:00 – 14:00	**Lunch**
14:00 – 14:20	Update on LC16m8 vaccine – **H. Yokote**
14:20 – 14:40	Update on smallpox vaccine IMVANEX® (IMVAMUNE®) – **N. Samy**
14:40 – 15:00	Progress towards approval and deployment of Arestvyr® (ST-246) – **D. Hruby**
15:00 – 15:20	Update on the development of Brincidofovir (CMX001) for smallpox – **R. Lanier**
15:20 – 15:50	**Tea/coffee break**
15:50 – 16:10	FDA perspective on the development and approval of smallpox medical countermeasures – **L. Borio**
16:10 – 16:30	EMA progress on antivirals – **E. Pelfrene**
16:30 – 16:50	Update variola virus repositories biosafety inspection visits – **K. Kojima**
16:50 – 17:30	General discussion
	CLOSE OF DAY ONE

DAY TWO – 13 January 2016

Closed discussion for members and Scientific Subcommittee

09:00 – 10.00	Implications of synthetic biology on future variola virus research
10:00 – 10:30	**Tea/coffee break**
10:30 – 11:30	Update the WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA
11:30 – 12:30	Future variola virus research priorities (vaccines, diagnostics, antivirals)
12:30 – 14:00	**Lunch**
14:00 – 15:00	Finalization of draft ACVVR report
	CLOSE OF MEETING

ANNEX 3

List of participants

Seventeenth meeting of the Advisory Committee on Variola Virus Research

12 and 13 January 2016

Salle D, WHO headquarters

Geneva, Switzerland

MEMBERS OF THE ADVISORY COMMITTEE

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***** Did not attend

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Dr David Ulaeto, Department of Biomedical Sciences, Defence Science and Technology Laboratory, Porton Down, Salisbury SP4 0JQ, UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

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Dr Hiroyuki Yokote, Regulatory Affairs, The Chemo-Sero-Therapeutic Research Institute, 1314-1 Kyokushi Kawabe, Kikuchi-si, Kumamoto 869-1298, JAPAN

WORLD HEALTH ORGANIZATION

REGIONAL OFFICES
Dr Eugene Gavrilin, CDS Labnet Co-coordinator, Representative of the Regional Office for Europe

HEADQUARTERS
Dr Bruce Aylward, Executive Director ad interim for Outbreaks and Health Emergencies and Special Representative of the Director-General for the Ebola Response
Dr Sylvie Briand, Director, Pandemic and Endemic Diseases

* Did not attend.

Dr Marc Sprenger, Director, Antimicrobial Resistance Secretariat, Office of the Director-General
Dr Ana Maria Henao Restrepo, Medical Officer, Immunization, Vaccines and Biologicals
Mr Alejandro Costa, Team Lead, Pandemic and Epidemic Diseases
Dr Kazunobu Kojima, Global Capacities, Alert and Response
Mrs Anne Mazur, Principal Legal Officer, Office of Legal Counsel
Dr Asheena Khalakdina, Technical Officer, Pandemic and Endemic Diseases
Mr David FitzSimons, Rapporteur

ANNEX 4

Terms of reference of the Advisory Committee on Variola Virus Research

In compliance with resolution WHA52.10, adopted by the Fifty-second World Health Assembly on 24 May, 1999, a group of experts has been convened to

- "establish what research, if any, must be carried out in order to reach global consensus on the timing for destruction of existing variola virus stocks", and
- commence, if appropriate, the development of "a research plan for priority work on the virus".

Over a longer term, this group of experts will also be expected to:

- "advise WHO on all actions to be taken with respect to variola
- devise a mechanism for reporting of research results to the world health community
- outline an inspection schedule to confirm the strict containment of existing stocks and to assure a safe and secure research environment for work on the variola virus.. .".

The first meeting of this group of experts, to be called the **WHO Advisory Committee on Variola Virus Research,** will be from 6 to 9 December, 1999, at WHO headquarters in Geneva. The committee will carry out its deliberations under the leadership of a Chairman elected from among the members of the Advisory Committee. The presentations to the committee and its deliberations will be collated and recorded by a Rapporteur.

The members of the committee will be required to apprise themselves of the current state of knowledge about variola virus, and the arguments concerning the desirability of conducting further research on it. In order to assist the group of experts to obtain an overview on these topics, a series of formal presentations and discussions has been organized. It will be the duty of the Chairman to keep presentations and discussion to the time allotted in the agenda, and to moderate the discussion. In the light of the information presented and the relevant discussion, the members of the Advisory Committee will be expected at the end of the third day to make a recommendation to the Director-General of WHO on whether or not further research is required. If the committee reaches a decision by consensus, this decision will be recorded by the Rapporteur, along with a summary of the supporting reasons. Should the committee be unable to reach a consensus, the Rapporteur will record the majority and dissenting views, again with a summary of the supporting reasons.

Should the decision be taken that further research on variola virus is required, the Committee members must then begin to develop the research plan, and the procedures for organising and monitoring the necessary work and communication of results. It may be necessary to appoint a scientific sub-committee or steering committee to facilitate these activities. The members of this sub-committee would be appointed by WHO in consultation with the Chairman and could include some members drawn from the Advisory Committee on Variola Virus Research and may include additional specialists selected to complete the scientific expertise required on the sub-committee.

ANNEX 5

WHO Recommendations concerning the distribution, handling and synthesis of variola virus DNA

Based upon recommendations made to WHO bythe WHO *Ad Hoc* Committee on Orthopoxvirus Infections (1990 and 1994) and the WHO Advisory Committee on Variola Virus Research (2003, 2004 and 2007)

Revised 13 January 2016 (by WHO Advisory Committee on Variola Virus Research at its seventeenth meeting)

Preamble

The only known stocks of variola virus are held at the two global repositories: the US Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America, and the State Research Center of Virology and Biotechnology (Vector), Koltsovo, Novosibirsk Region, Russian Federation, both of which are World Health Organization (WHO) Collaborating Centres[16]. Any research using live variola virus has to be performed in the maximum containment laboratories of these institutions and requires prior permission from WHO.

Genetic engineering of variola virus and attempts to produce live variola virus from DNA are strictly prohibited.

The purpose of these recommendations is to prevent the reconstruction of variola virus or the construction of a virus which will cause a disease with the same attributes as smallpox, either through the reactivation of variola virus DNA or the incorporation of variola virus DNA sequences into other orthopoxviruses. There are several ways to achieve this goal; one is to limit the amount of variola virus DNA held by any one laboratory to an amount far less than a complete genome; another is to institute operations and practices that preclude any possibility of variola virus DNA coming in contact with another replication-competent orthopoxvirus. As the capacity to synthesize genes has improved to the point where genes and whole genomes can be created from sequence information, it is important to remember that DNA encoding variola virus proteins should also be handled with these restrictions in mind.

In its naked form, variola virus DNA is not infectious. Scientists wishing to perform research on individual variola virus genes may obtain parts of the variola virus genome from one of the two WHO Collaborating Centres for Smallpox. Alternatively, researchers may wish to synthesize or purchase such DNA directly. WHO or the WHO Collaborating Centres will advise scientists on the procedure to follow in order to obtain permission to receive or synthesize variola virus DNA. Scientists should be aware that the amount of DNA they request or hold must not exceed 20% of the total variola virus genome (see also below).

The scientific community must be fully aware that the distribution, synthesis and handling of variola virus DNA is governed by a series of recommendations made initially by the WHO *Ad Hoc*

[16] WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA (Vector) and WHO Collaborating Centre for Smallpox and Other Poxvirus Infections (CDC).

Committee on Orthopoxvirus Infections and updated by the WHO Advisory Committee on Variola Virus Research. These recommendations have been endorsed by the World Health Assembly. WHO's recommendations are intended to be incorporated into individual Member States' biosafety guidelines or legislation. Scientists must also comply with the requirements of local guidelines or legislation if they wish to obtain, handle or synthesize variola virus DNA. The present document gives an overview of these recommendations, which are reproduced in their original wording as found in the various WHO meeting reports (http://www.who.int/csr/disease/smallpox/research/en/index.html).

Distribution of variola virus DNA

The two WHO Collaborating Centres, acting as repositories for variola virus, may distribute variola virus DNA fragments to appropriate research laboratories that request them provided that:

a) The request has been submitted to the international repository through the WHO Secretariat (*1, 2*).

b) The receiving laboratory agrees that the DNA will not be distributed to any third party unless authorization by WHO has been obtained. This must be controlled through a Material Transfer Agreement between the distributing and receiving laboratories (with copy to WHO) (*1, 2, 3*).

c) The receiving laboratory must submit an annual report on the status of the variola virus DNA to WHO, and if appropriate, the WHO Collaborating Center (*2*).

No laboratory, other than the designated smallpox WHO global repositories, shall be permitted to hold variola virus DNA representing more than 20% of the variola virus genome at any one time (*2*).

Fragments of variola virus DNA for diagnostic kits, not exceeding 500 base pairs in length, may be freely distributed for use as positive controls or standards in diagnostic kits, providing collectively they do not exceed 20% of the total genome size held by any entity (*4, 5*).

Handling of variola virus DNA

Studies on variola virus DNA are permitted on conditions that:

a) The DNA will not be used for insertion into other poxviruses (*2*).

b) All work with variola virus DNA can be done only following approval of a written risk assessment by the appropriate local safety committee (*2*).

c) No other orthopoxviruses are handled in the laboratory rooms where variola virus DNA is present (*2*).

d) All by-products containing variola virus DNA must be disposed of at the conclusion of the work by autoclaving at 120°C for 30 minutes (*6*).

Synthesis of variola virus DNA

a) Attempts to synthesize full-length variola virus genomes or infectious variola viruses from smaller DNA fragments are strictly forbidden (*7*).

b) Synthesis of variola virus DNA to express a variola virus protein, or synthesis of codon-modified DNAs for the same purpose requires prior permission from WHO via the Chair of the ACVVR Scientific Sub-Committee. Similarly, mutagenesis of orthopoxvirus DNA with the aim of producing a variola virus protein requires prior permission from WHO through the same channel. Those undertaking synthesis of variola virus DNA are under the same obligations and constraints outlined above in the section on Distribution of variola virus DNA.

c) Under no circumstances can any single laboratory other than the designated WHO Collaborating Centres hosting the variola virus repositories hold DNA comprising more than 20% of the total genome (*4*, *7*).

d) The only exception to c) is the production of DNA microarrays, on which small oligonucleotides (less than 80 base pairs) are covalently bound to a matrix and which, in aggregate, may span the entire variola virus genome, does not require permission from WHO (*4*, *5*).

e) For diagnostic kit purposes, variola virus DNA fragments up to 500 nucleotides may be synthesized without notification to WHO.

These policies also extend to the manufacturers of synthetic DNA who must also be held responsible for upholding these recommendations and national policies.

Reporting obligations

Variola virus DNA is distributed to scientists on the understanding that an annual report on the status of variola virus DNA clones will be made to the international repository (see above: distribution of variola virus DNA, paragraph c). This reporting obligation also applies to scientists who have obtained permission from WHO to synthesize variola virus DNA larger than 500 base pairs or generate variola virus-like DNA by site-directed mutagenesis of other orthopoxvirus DNA.

Definition of variola virus DNA

Variola virus DNA is defined as any DNA sequence of any length:

- unique to variola virus, and/or
- which encodes one or more variola virus polypeptides.

A variola virus polypeptide is defined as any polypeptide of any length that contains amino acid sequences that are unique to variola virus.

References

1. Report of the *Ad Hoc* Committee on Orthopoxvirus Infections, 1990, page 5.
2. Report of the *Ad Hoc* Committee on Orthopoxvirus Infections, 1994, page 8.
3. Report of the WHO Advisory Committee on Variola Virus Research, 2007, 23.4
4. Report of the WHO Advisory Committee on Variola Virus Research, 2003, 11.7
5. Report of the WHO Advisory Committee on Variola Virus Research, 2004, 8.2.
6. Report of the Ad Hoc Committee on Orthopoxvirus Infections, 1994, page 9.
7. Report of the WHO Advisory Committee on Variola Virus Research, 2004, 8.4.