

WHO Advisory Committee on Variola Virus Research

Report of the Twelfth Meeting

Geneva, Switzerland
17–18 November 2010

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Table of Contents

1. Report from the Secretariat	3
2. Update on WHO-approved research proposals	4
3. Update on variola virus DNA clones held at NICD, South Africa	4
4. Scientific Review of Variola Virus Research 1999–2010	5
5. The Advisory Group of Independent Experts to review the smallpox research programme (AGIES)	9
6. Smallpox diagnostic network	10
7. Update on variola virus stocks held in the repositories in the United States and the Russian Federation	11
8. Update on anti-orthopoxvirus therapeutics	11
9. Update on animal models	12
10. Update on smallpox vaccines	13
11. WHO smallpox vaccines stocks	15
12. WHO Smallpox Eradication Programme Archives	15
13. Future of the Advisory Committee	15
Annex 1. Summaries of the presentations	17
Annex 2. Agenda	48
Annex 3. List of participants	51

1. Report from the Secretariat

- 1.1. The WHO Advisory Committee on Variola Virus Research met on 17 and 18 November 2010 with Professor G.L. Smith as Chairman and Drs R. Drillien and F. McLellan as Rapporteurs.
- 1.2. Dr K. Fukuda opened the proceedings, noting that discussions on these issues have been ongoing since 1986 and remain of great interest to countries. Among other items for discussion, this group has been assembled to assess a major review of research related to variola virus, in advance of a discussion to take place at the Sixty-fourth World Health Assembly on the timing of the destruction of variola virus stocks. The assessment will consider two key items: firstly, a review of the literature and unpublished data conducted by a group of scientists endorsed by this committee; and secondly, an external review of the review itself, which has been conducted by experts outside the variola virus field. This meeting will produce a meeting report in addition to the other two documents, all of which would be made available as soon as possible prior to being submitted to the Executive Board in January 2011.
- 1.3. Dr P. Formenty updated the group on this year's activity in the WHO Smallpox Project. The report of last year's meeting of the Advisory Committee was noted by the World Health Assembly in May 2010. The report of this 12th meeting will be submitted to the Sixty-fourth World Health Assembly in May 2011. In May 2007, a major scientific review was called for in resolution 60.1; later in 2008 it was decided that this review would consist of two parts – the scientific review itself and an independent review of it by external experts outside the field of smallpox (the Advisory Group of Independent Experts to review the smallpox research programme, or AGIES, report). The inspection report of the WHO Collaborating Centre at Centers for Disease Control and Prevention, United States of America, was finalized last year and a report of the WHO Collaborating Centre at VECTOR (Novosibirsk, Russian Federation) is now being finalized. The work of the subcommittee on the Smallpox Laboratory Network has begun under the leadership of Dr J.-C. Piffarretti as coordinator. Standard Operating Procedures have been developed for the smallpox vaccine stockpile. The Archive project, which consists in part of scanning all the documentation related to smallpox, some 700 000 pages of searchable documents, was presented later in the meeting.
- 1.4. In line with WHO policy all members, advisers and observers of the Advisory Committee have completed and signed a Declaration of Interests. Six experts have declared a potential conflict of interest in the subject matter of this meeting. No relevant conflict of interests has been declared by the other experts. The declared interest reported by Peter Biggins and Jean-Claude Piffaretti were assessed to be minimal and unlikely to affect, or to be reasonably perceived to affect, their judgment. Four experts declared interests that the Secretariat has determined should be disclosed.

- Dr Jacob Thorup Cohn stated that he is employed by Bavarian-Nordic, a leading Danish privately held biotech firm in the field of smallpox countermeasures (antivirals, vaccines).
- Dr Randall Lanier stated that he is employed by and owns stock in Chimerix which is involved in the development of a product that may serve as a biodefense countermeasure in the event of a smallpox release. Dr Lanier further indicated that Chimerix has provided funding for his travel and subsistence for attendance at the current Advisory Committee meeting.
- Dr Grant McFadden stated that he had acted as a consultant for SIGA Corporation concerning their application to the FDA for drug approval of the ST-246 as an antiviral against smallpox.
- Dr Robert Drillien indicated that he has been a consultant for Bavarian-Nordic, a company producing a smallpox vaccine and that he was consultant for French Army on smallpox vaccine.

2. Update on WHO-approved research proposals

- 2.1 Dr R. Drillien gave an update on research proposals submitted to WHO and approved by the scientific subcommittee between November 2009 and August 2010. He noted that all the approved proposals are continuations of ongoing projects, not new proposals. Details of the projects are summarized in Annex 1. He briefly reviewed each proposal. The Committee asked the Secretariat to prepare a list of all research projects that have been concluded.

3. Update on variola virus DNA clones held at NICD, South Africa

- 3.1 Professor R. Swanepoel gave an update on the variola virus DNA clones held at the National Institute for Communicable Diseases (NICD), which is the successor to the National Institute of Virology, South Africa. After the declaration by WHO of the eradication of smallpox in 1980, all variola virus national collections were to be stored in four repositories located in the USA, the USSR/Russian Federation, South Africa, and the United Kingdom. In 1982 the variola virus stocks held in the United Kingdom were transferred to the United States. An agreement was reached that South Africa would be given the clones of recombinant plasmids containing variola virus DNA fragments that had been prepared in the United Kingdom by Dr K.R. Dumbell in exchange for destroying their stock. On 9 December 1983 the variola virus stocks were destroyed in the presence of Dr Dumbell who had been appointed by WHO to witness the destruction. NICD then received the non-infectious DNA clones of recombinant plasmids. The clones, which have never been used, are currently held, as of October 2010 in storage inside the BSL4 facility at NICD. The South African Department of Health has now decided that clones of recombinant plasmids potentially useful in producing diagnostic reagents, and constituting no more than 20% of the genome of the virus should be retained. The rest of the clones should either be transferred to the CDC repository or destroyed under the

supervision of WHO. If the CDC repository already has duplicates of the clones it would be better to destroy them in South Africa than to transport them.

COMMITTEE DISCUSSION: The Committee suggested that in the event that clones of recombinant plasmids containing variola virus DNA fragments are duplicated at CDC, there is no need for transfer of the stocks, nor for their retention.

4. Scientific Review of Variola Virus Research 1999–2010

- 4.1 The Chair thanked the authors of the Scientific Review of Variola Virus Research 1999–2010 for their work, and especially for their patience with editorial changes.
- 4.2 The overall assessment of the Committee was the following: the consensus view of the Committee on the conclusions reached in the review overall was that laudable progress has been made in all areas, while recognizing that additional science can be done. The past decade has seen a remarkable amount of output. Progress towards the goals for which the research was permitted has been exceptional but is not yet complete. The tasks for which live virus is needed have narrowed considerably.
- 4.3 Dr A. Alcamì presented Chapter 1, on smallpox vaccines, which is summarized in Annex 1. The chapter concluded that "licensure of smallpox vaccines grown in tissue culture has been a useful step forward; however, use of these vaccines would be medically contraindicated for individuals with immunodeficiency and certain dermatological conditions. Since smallpox has been eradicated, the efficacy of new generation vaccines will need to be tested using poxviruses related to variola virus in animal protection studies, and safety and immunogenicity studies in humans. However, confidence in the ability of these vaccines to protect against smallpox would be increased by use of live variola virus for in vitro neutralization tests and non-human primate studies."

COMMITTEE DISCUSSION: The Committee recalled that the smallpox vaccine was essential for the eradication of smallpox. Nevertheless, there remains a compelling need for vaccines with a better safety profile. Some progress has been made along these lines: for example, a vaccine grown in tissue culture to modern standards of good manufacturing practice and an attenuated vaccine have been produced and licensed, and more attenuated vaccinia virus vaccine strains are in development. There was considerable discussion about the relative merits of animal models using variola virus for further testing of vaccine efficacy of these vaccines in the absence of human disease. The Committee agreed that in vitro neutralization and non-human primate studies with live variola virus would be desirable for increased confidence in the efficacy of candidate vaccines. Some members felt that several cell culture-passaged attenuated vaccines may have reached a level of development that does not require further use of live variola virus.

DECISION: The Committee agreed with the conclusions of the authors.

- 4.4 Dr I. Damon presented Chapter 2, on laboratory diagnostics, which is summarized in Annex 1. The chapter conclusion is that "there has been a remarkable expansion

in the number of variola virus nucleic acid diagnostic assays, and a very limited expansion of immune or protein-based diagnostic techniques. All assays developed to date are research based; none have completed regulatory review and approval processes."

COMMITTEE DISCUSSION: The Committee discussed the clinical algorithm shown for the diagnosis of smallpox, noted the important considerations of predictive value positive in interpreting assay results for a low prevalence disease, and recommended this discussion be added to Chapter 2. The Committee noted that several organizations have produced videos and DVDs for diagnosis, including WHO, CDC, the US Army, other Member States, and others.

DECISION: There was divergent opinion within the Committee about whether live variola virus is needed for further development of, improvement in and licensure of diagnostics for clinical use.

- 4.5 Dr G. McFadden presented Chapter 3, on variola genomics, which is summarized in Annex 1. The chapter concluded that "publicly available genomic information has been used by many international scientists to design highly sensitive virus diagnostics. The newly-gained understanding of the relationship between variola virus and other orthopoxviruses also provides important clues to understanding the value and limitations of animal models for human smallpox. Remarkable expansion in the technologies of DNA synthesis, sequencing, and cloning has created today's situation, where it is now technically possible to synthesize the entire variola virus genome from scratch, using only publicly available sequence information, and to reconstitute infectious virus using currently available techniques of molecular biology. As a result of this ability, future biodefence strategies need to incorporate new thinking regarding how best to control the application of these synthetic biology technologies."

COMMITTEE DISCUSSION: The Committee noted the tremendous achievements of variola virus genome sequencing, and that 48 genomes have been sequenced, analysed and published. The Committee also recognized the change in landscape that occurred with the advent of synthetic biology, and the realization that a live variola virus could be created without access to current WHO Collaborating Centre stocks of variola virus. It was also noted that it was potentially possible to create a synthetic variola virus that would be undetectable by some current diagnostics – though whether this would result in a pathogenic virus is unknown.

The Committee stressed the need for further regulatory measures, revised guidelines, and international adoption of rules to reduce the likelihood that live variola virus will be created. The Committee debated whether recombination between orthopoxviruses that exist in nature could result in a virus with similar virulence to variola virus and recognized the uncertainty in this regard. Questions were asked about the insertion of foreign genes that would change virulence, which was briefly mentioned in this chapter. It was noted that all genetic engineering of variola virus, including the insertion of foreign genes into variola virus, is prohibited. The host-specificity of variola virus was discussed. It was noted that the

reason why variola displays human-specific tropism remains unknown and that this is an important question about many pathogens.

DECISION: The Committee agreed that there was no further need for live variola virus for the study of variola virus genomics.

- 4.6 Chapter 4, on the status of WHO Collaborating Center repositories, was presented by Dr I. Damon and is summarized in Annex 1. This chapter summarizes the current status (as of January 2010) of live variola virus repositories, variola virus DNA stocks, and – where appropriate – use and distribution of variola gene fragments as per WHO recommendations. The repositories of variola virus are currently restricted to two laboratories: the WHO Collaborating Centre on Smallpox and other Poxvirus Infections at the Centers for Disease Control and Prevention in Atlanta, Georgia, USA; and the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at the State Research Center of Virology and Biotechnology VECTOR (SRC VB VECTOR) in Koltsovo, Novosibirsk Region, Russian Federation. Annual reports from these two laboratories, regarding use of live variola virus and the status of the repositories, are submitted to the WHO Secretariat. Since 2000, these reports have also been made in person at the annual meetings of the WHO Advisory Committee on Variola Virus Research, which are convened to review work with live variola virus. Abstracts of these presentations are available online, via the WHO web site.¹

COMMITTEE DISCUSSION: The Committee noted that for more than two decades variola virus has been contained successfully in two secure locations, during which it has been used for WHO-sanctioned research by international partners. In addition, non-infectious variola virus DNA fragments have been distributed in accordance with WHO guidelines and under the supervision of the WHO Secretariat.

DECISION: The Committee agreed with the views of the authors.

- 4.7. Dr P. Jahrling presented Chapter 5, on animal models and pathogenesis, which is summarized in Annex 1. Dr Jahrling concluded that it is likely that no single combination of conditions will result in a model which will simultaneously satisfy all of the criteria required under the United States Food and Drug Administration (US FDA) ‘Animal Rule’ (US 21CFR310.610); different models may be required to assess different indications. Further refinement of the primate models might include pathophysiologic data from studies using telemetry and medical imaging. While a significant proportion of this developmental work can be accomplished using surrogate orthopoxvirus in rodents and primates, increased confidence in countermeasures against variola virus can be obtained only by efficacy testing in primate models using variola virus.

COMMITTEE DISCUSSION: The Committee noted the considerable progress in the development and application of models of variola virus infection that reflect some aspects of human smallpox. These models have been used for understanding

¹ <http://www.who.int/csr/disease/smallpox/research/en/index.html>, last accessed, 1 December 2010

pathogenic mechanisms, evaluation of new antiviral drugs and development of diagnostic tools. In addition, a primate monkeypox model has been used for the evaluation of candidate vaccines and antiviral agents.

The evolving requirements of regulatory authorities, and the underpinning regulatory science required for licensure, were discussed and the extensive dialogue between researchers and regulatory agencies was noted. This dialogue has helped shape experimental programmes and is ongoing. It is expected that protective efficacy against variola virus in animal models will be needed for licensure of new antivirals for smallpox.

DECISION: The Committee believes that animal work with live variola virus has strengthened the scientific justification for licensure of antiviral agents, and some members of the Committee believe that additional studies with live variola virus are necessary.

- 4.8 Dr J. Huggins presented Chapter 6, on antiviral agent development for smallpox treatment, which is summarized in Annex 1. The project described in this chapter was undertaken in order to obtain two approved oral antiviral compounds, with different mechanisms of action, which can treat clinical cases of smallpox. It is remarkable that three compounds (cidofovir, ST-246[®] and CMX001) that inhibit variola virus replication, in cell culture and in multiple animal models utilizing surrogate orthopoxvirus models, have gained Investigational New Drug status from FDA for treatment of orthopoxvirus infections. Initial human studies are underway. Since variola virus has been eradicated from the human population, traditional clinical efficacy trials are not feasible. In addition, it is not possible to conduct ethical clinical trials in humans, so demonstration of efficacy must utilize the FDA ‘Animal Rule’. Given the uncertainties in that rule, and the fact that there is currently no antiviral agent approved for any type of smallpox indication (treatment or chemoprophylaxis), it is difficult to provide firm estimates concerning timelines or the data required for approval.

Dr Huggins concluded that it could be argued that the capability to perform work with live variola virus must be maintained until an adequate number of antivirals, with different mechanisms of action, have gained regulatory approval and could be used worldwide to combat an outbreak.

COMMITTEE DISCUSSION: The Committee applauded the development of two extremely promising antivirals (ST-246[®] and CMX001) that have demonstrated protection in several animal models of orthopoxvirus infection, including variola virus, monkeypox virus, and rabbitpox virus. Phase 1 and 2 clinical studies have also demonstrated an excellent safety profile for the two leading candidate compounds. The Committee noted that some animal data, especially from studies of ST-246[®], showed that vaccination and simultaneous drug administration can be performed without attenuation of the immune response. It has also been shown that administration of both antivirals simultaneously provided greater protection in some models than either drug alone. Additional antivirals, besides ST-246[®] and CMX001, are in earlier stages of evaluation. The Committee emphasized that the question for regulatory agencies is what additional data are needed for licensure. It

was noted that the requirements for clinical trials are very demanding and are not necessarily within the possibilities of the biological systems available. The Committee debated whether primary data could be assembled and then given to regulatory agencies for feedback. It was concluded that there are differences between various regulatory agencies and that they would most likely give non-binding scientific advice.

DECISION: The Committee agreed that data using live variola virus have facilitated progress towards licensure of the antiviral agents, but there was divergence of opinion among the Committee members about whether additional animal model data with live variola virus should be required for completion of licensure in all countries.

5. The Advisory Group of Independent Experts to review the smallpox research programme: comments on the Scientific Review of Variola Virus Research, 1999–2010

- 5.1 Dr T. Sorrell presented the report of the Advisory Group of Independent Experts to review the smallpox research programme (AGIES), which is summarized in Annex 1. She noted that some minor inaccuracies in the report had come to light, and that these would be corrected. The Advisory Group considered the Scientific Review of Variola Virus Research, 1999–2010 to be clearly written and comprehensive, and to provide an accurate and up-to-date review of variola virus research, including the impact of regulatory restrictions on current and future research. The full report is presented in three sections, in line with the terms of reference provided to the Group. Section 1 provides a summary of each chapter of the Scientific Review, followed by specific comments. Section 2 contains the Group's recommendations for further research and comments on variola virus repositories. Section 3 summarizes the Group recommendations for ensuring high standards of security related to re-emergence of smallpox.

COMMITTEE DISCUSSION: The Committee considered and discussed the Advisory Group's report, which was considered first class. The Committee also welcomed the process by which the views of the Advisory Group report were provided to the authors of the Scientific Review, and thanked the authors for their work. It was noted that there were a few minor inaccuracies in the report; the members of the AGIES in attendance acknowledged these and agreed to rectify them. A finalized report will be tabled.

- 5.2 The Committee discussed timeframes for the storage of variola virus DNA fragments in laboratories other than those at CDC and VECTOR, the requirement that these DNA fragments and other reagents must be destroyed when the experiments are completed, and whether confirmation of destruction exists from laboratories that have received DNA samples. It was suggested that the WHO Secretariat contact all recipients to clarify these issues.
- 5.3 The Committee discussed that several thousand compounds have been screened for activity against orthopoxviruses and variola virus, utilizing an in vitro cell culture

based assay. The structure of these compounds will not be revealed until late in development. The results of screening assays were reported to the Advisory Committee at its 2nd, 3rd and 5th meetings in 2001 and 2003. Many of the compounds initially evaluated against variola virus were identified by a supplier identification number rather than being described by chemical class. The structure of some compounds were revealed when they were evaluated in the variola animal models. The Secretariat noted that, in accordance with the relevant resolutions of the World Health Assembly, the proposals, outcomes and benefits resulting from this research should be made available to all Member States. It was also noted, however, that it may be difficult for the Collaborating Centres to gain access to the compounds, and for companies to invest in developing promising compounds, without non-disclosure agreements or similar forms of protection. It was also noted that release of data showing that many compounds did not have activity against variola virus would prevent additional studies being done by others with the same compounds.

- 5.4 The Committee discussed development of new compounds, and whether further large-scale screening for additional compounds is necessary, in view of the existence of two compounds, each with different targets, that are effective at inhibiting disease caused by several orthopoxviruses, including variola virus in several animal models. Concerns were expressed about the potential engineering of variola virus strains that are resistant to these drugs and the fact that neither drug has been approved by any regulatory agency, creating a complicated scenario for a policy on utilization.
- 5.5 Whether a third compound should be developed was discussed. The Advisory Group's authors noted that they did not recommend that additional antivirals should be pursued immediately, but they had raised a question about the importance of resistance and noted the need for expert consultation on whether further antivirals are needed.

6. Smallpox diagnostic network

- 6.1 Dr J.-C. Piffaretti presented an update on the work of the subcommittee of the Smallpox Laboratory diagnostic Network (SLN) (see summary in Annex 1). The subcommittee has been formed to discuss the establishment of a WHO network of high-level diagnostic laboratories throughout the world with the aim of detecting rapidly and consistently any emergence of variola viruses. The features of the SLN are in the process of being finalized. The SLN would comprise the two reference laboratories together with a number of regional laboratories, one or two in each WHO region.

COMMITTEE DISCUSSION: Concerns were expressed about the long list of criteria the regional laboratories would have to meet, but it was noted that existing high-level medical diagnostic laboratories would already have satisfied most of the criteria. There was extensive discussion about financing, the use of existing laboratories and trained personnel, integration into national surveillance systems, and whether to structure the network as part of national capacity strengthening under the International Health Regulations (2005). The WHO Secretariat clarified

that the network is intended for a smallpox event only, to generate actionable results, with a clear plan for mobilization if there are positive results.

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

- 7.1. Dr K. Karem gave an update on the WHO Collaborating Centre repository at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA (see summary in Annex 1). Dr Karem described the recent work on the evaluation of the variola-antigen capture assay, and the recent work, post-sequencing of additional cowpox virus genomes, discovering that some of the “variola specific” PCR assays have now lost specificity for variola due to additional sequence data. Dr Karem presented a study that demonstrated that variola specific monoclonal antibodies favour gamma-irradiated antigen. Detection of live variola virus antigen is at least four times less sensitive than gamma-irradiated material. This disparity was due neither to virus strain differences nor due to artificial reactivity after gamma-irradiation. Testing of other methods of inactivation have been performed (UV irradiation, heat, formalin) and have been found to be inferior to gamma irradiation, reacting in a manner similar to live antigen.

COMMITTEE DISCUSSION: The need for more sequence data from other orthopoxviruses was discussed in relation to the specificity of variola virus nucleic acid based diagnostics. There was also discussion about the best method for confirmation if a case of smallpox was found. The need for a simpler assay where real-time PCR is not available was noted.

- 7.2. Dr S. Shchelkunov reported on the variola virus collection of the WHO Collaborating Centre repository in VECTOR, Russian Federation, (see summary in Annex 1). In 2010, the glass vials in which variola viruses were stored in a frozen condition were replaced for safety reasons by polypropylene cryovials with printed labels on them that are resistant to disinfectant solutions. Current research activity included the testing of the antiviral properties of compounds with previously identified antiviral efficacy against other orthopoxviruses, and the testing of the neutralizing properties of single-chain antibodies with previously identified neutralizing activity against other orthopoxviruses.

COMMITTEE DISCUSSION: Questions were raised about the nature of the antivirals used. This information will be provided in the annual report to WHO.

8. Update on anti-orthopoxvirus therapeutics

- 8.1 Dr V. Olson gave an update on the use of live variola virus to evaluate antivirals against variola virus (see summary in Annex 1). Dr Olson provided data demonstrating that an extract from the plant *Sarracenia purpurea* was effective at restricting the replication of variola virus and vaccinia virus and seemed to have the same mechanism of action against both viruses. This activity against orthopoxviruses indicated its potential use as a therapeutic agent. Future work will focus on further characterization of the active component of the crude extract

component (which may necessitate further anti-variola evaluation), and its potential efficacy against systemic disease caused by an orthopoxvirus in an animal model.

COMMITTEE DISCUSSION: Discussion took place about reductions in virus titre, and the effect of the compound on other viruses. It was noted that the presence of the extract prevented virus transcription in a dose-dependent manner but the active component of the extract is still unknown and is being sought by biochemical fractionation. Whether the compound will be useful from a practical perspective will be elucidated through future (animal) studies.

- 8.2 Dr D. Hruby gave an update on the development of the antiviral compound ST-246[®] (see summary in Annex 1). The compound has shown potency against all orthopoxviruses tested in cell culture and in animal models and these studies have included variola virus and monkeypox virus in primate models. In addition, it has shown good stability and an excellent safety profile in all tests conducted. Phase II clinical trials in animals have been completed along with the New Drug Application enabling toxicology studies. The pharmaceutical company SIGA is in the midst of commercial manufacturing and preparation for the pivotal safety and efficacy studies. While the appropriate human dose is still being studied, Dr Hruby concluded that ST-246[®] is in late stage development and procurement-ready.

COMMITTEE DISCUSSION: There was discussion about whether regulatory agencies will require further experiments with live variola virus to support licensure, particularly studies in primates to be sure that the model used represents the human situation as far as possible. Questions were raised about the possibility of undertaking clinical trials for compassionate use. The appropriate human dose is still being studied.

- 8.3 Dr R. Lanier gave an update on the development of Chimerix's smallpox antiviral CMX001 (see summary in Annex 1). CMX001 is a lipid conjugate of the acyclic nucleotide phosphonate, cidofovir. CMX001 has a broad spectrum inhibition of double-stranded DNA viruses that cause human disease, a high genetic barrier to resistance, convenient oral administration as a tablet or liquid, and no evidence to date of renal toxicity. It shows efficacy against orthopoxviruses in cell culture systems and in animal models. CMX001 is in development under the FDA 'Animal Rule' and in phase II clinical development in humans for treatment of cytomegalovirus infections.

COMMITTEE DISCUSSION: Plans for additional studies in several species were discussed. The Committee noted that dialogue between researchers and regulatory agencies is ongoing. Whether regulatory agencies should be invited to future meetings of this Committee was discussed.

9. Update on animal models

- 9.1 Dr P. Jahrling reported on perspectives on the development of primate models for evaluating countermeasures against human smallpox and monkeypox (see summary in Annex 1). Dr Jahrling explained that since 1999, some progress has been made in

developing animal models, but emphasized that there is still no animal model that satisfactorily recapitulates all relevant aspects of human smallpox. He presented new studies on the pathology of human smallpox in comparison with cynomolgus macaques challenged intravenously with variola and monkeypox viruses. His conclusion was that significant progress has been made, but more work with live variola virus remains to be done.

COMMITTEE DISCUSSION: There was extensive discussion as to whether monkeypox virus or variola virus in monkeys is a better human smallpox model. It was concluded that neither model completely recapitulates human smallpox, but both models may be used to evaluate diagnostic, antiviral and vaccine efficacy. Some Committee members believe that a variola virus challenge model would be essential for licensure of countermeasures. There was divergence of opinion within the Committee as to whether additional animal models should be sought, or whether the current models have provided sufficient data to enable evaluation of new vaccines and antivirals.

10. Update on smallpox vaccines

- 10.1 Dr L. Wegner presented the clinical development status of the live attenuated smallpox vaccine IMVAMUNE® (see summary in Annex 1). IMVAMUNE® has been proven to be safe in healthy individuals and in HIV-infected patients with CD4+ T-cell counts in excess of 200 per μ l. IMVAMUNE® induces a rapid and strong vaccinia-specific immune response comparable between healthy subjects and at-risk groups.

COMMITTEE DISCUSSION: Questions were raised about T-cell response in comparison with traditional vaccines. Dr L. Wegner informed the Committee that IMVAMUNE® has a T-cell response that is comparable with that of the traditional vaccines. The number of doses required was discussed as was the route of administration (at present subcutaneous administration is superior to intramuscular). There was a discussion around the challenges that IMVAMUNE® faces under the ‘Animal Rule’ and it was noted that although IMVAMUNE® is considered safe for human trials, any comparison with traditional vaccines (1st and 2nd generation) is not possible as the US-FDA will not allow trials using the traditional vaccines because of safety concerns. The upcoming phase III trial will be focused on safety and immunogenicity and will be designed in collaboration with the US-FDA. Results are awaited with great interest as IMVAMUNE® seems to be the most advanced product under the ‘Animal Rule’. Adverse effects were discussed and the lack of cardiac toxicity was emphasized. The advantages of the MVA strain safety profile in an emergency setting were discussed together with the relative advantages or hurdles of the different routes of administration. The potential advantages that IMVAMUNE® offers to existing stockpiles and immune compromised populations, was also discussed. A freeze-dried version is foreseen. The price of the vaccine, which is higher than traditional vaccines, was discussed. It was acknowledged that that price will present a specific challenge for the developing world but that it is anticipated that the price will decrease. Shelf-life is anticipated to be at least three years in its current formulation; a significant longer shelf-life requires a freeze-dried preparation. Data on vaccination with

IMVAMUNE® in HIV infected individuals with low CD4+ T-cell counts were discussed as well as the data from the non-human primate studies.

- 10.2 Dr H. Yokote presented an update on the use of smallpox vaccine LC16m8 in Japan, (see summary in Annex 1). In the 1960s, a Smallpox Vaccine Research Committee was convened in Japan for the research into smallpox vaccine, resulting in the establishment of a new attenuated smallpox vaccine: LC16m8. The LC16m8, an attenuated tissue-cultured vaccine, was licensed in Japan in 1975. Recently, the Smallpox Vaccine Research Group has been convened to address research of medical countermeasures against bioterrorism. This Group has demonstrated that LC16m8 has (i) as much efficacy as the Lister strain vaccine or the NYCBH strain vaccine that eradicated smallpox and (ii) has a higher level of safety compared with the Lister strain vaccine or the NYCBH strain vaccine. The post-marketing surveillance study results indicated that LC16m8 has a high safety and immunogenicity profile for both vaccinia-naïve and experienced subjects along with the findings in the previous clinical researches. Dr Kurane indicated the interest of the Smallpox Vaccine Research Group to evaluate post-LC16m8-vaccination sera for anti-variola neutralization capacity, and the interest in collaborating with the WHO Collaborating Centres on this study.

COMMITTEE DISCUSSION: Questions were raised about the enrolment of immunocompromised patients and about the number of people with atopic dermatitis who were vaccinated. The Committee asked about national plans for stockpiling this vaccine and also for making it available as part of the global stockpile. The official policy of the Japanese Government concerning the national stockpile is not currently publicly available. The maximum manufacturing capacity of 80 million doses yearly was confirmed.

- 10.3 Dr I. Damon presented the use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines, (see summary in Annex 1). Dr Damon presented the role of variola virus plaque reduction neutralization test as a marker for smallpox vaccine efficacy; the importance of such evaluations are perhaps greatest for the evaluation of these vaccines that do not elicit a “take”, the traditional measure of vaccine success. Dr Damon presented data that suggested there was only a weak correlation between vaccinia and variola neutralization data (noted by Dr Banerjee to be consistent with Dr Downie’s earlier investigations). Dr Damon also presented data showing that ACAM3000 MVA is safe and well tolerated at all doses and routes, is immunogenic in eliciting anti-vaccinia neutralizing antibody and T-cell responses and anti-variola neutralizing antibody responses. The correlation between peak anti-variola Nab response, decreased response to Dryvax, and associated duration of vaccinia shedding, is being further analysed as a surrogate marker of efficacy.

COMMITTEE DISCUSSION: The Committee discussed the number of variables in the experimental set-up (e.g. viruses were grown in different labs) and whether these confound the results. An additional control was suggested. It was asked, but not answered, whether it is necessary to neutralize variola virus in vitro in order to qualify new vaccines.

11. WHO smallpox vaccines stocks

11.1 Dr P. Formenty presented an update of the WHO smallpox vaccines stocks (see Annex 1). WHO has built a strategic stock of smallpox vaccines of 30.5 million doses stored in Switzerland. Nearly all (98%) (ACAM2000™ smallpox vaccine - 30 million doses donated by Baxter) of the WHO strategic stock of smallpox vaccines is second-generation vaccine. The remaining (2%) (vaccinia - 530 000 doses from Belgium, Germany, Iran (Islamic Republic of), the Netherlands and the Russian Federation) of the WHO strategic stockpile is first generation vaccine. In addition, through a virtual stockpile mechanism, four Member States have pledged another 27 million doses to WHO in case of additional needs: France, Germany, New Zealand and USA. WHO has agreed Standard Operating Procedures with all four countries.

COMMITTEE DISCUSSION: The Committee asked whether there are plans to add this information to the WHO web site. The WHO web site will be re-launched at the end of 2010, at which time the information will be added.

11.2 The WHO Secretariat discussed global stockpiles and the prospect of mass vaccination campaigns. The distribution of antivirals during the recent H1N1 influenza pandemic was described. Within 10 working days, 80% of least-developed countries had received antivirals distributed from the stockpiles. Scenarios not driven by public health priorities but by security concerns are most problematic for the Organization. Direct discussions with security counterparts are needed.

12. WHO Smallpox Eradication Programme Archives

12.1 Ms M. Villemin presented an update on the digitization of the Smallpox Eradication Programme archives: outcomes, perspectives and strategy, (see summary in Annex 1). The digitization project for the Smallpox Eradication Programme archives started in June 2009. The preservation of the paper files and the integration of the scanned archives into a dedicated database with a powerful search engine have been achieved. After the integration and preservation, the next goal is to make all data available worldwide via a dedicated web interface.

COMMITTEE DISCUSSION: The Secretariat publicly thanked the presenter and her team for a monumental task of documentation and preservation. Most WHO Regional Office archives have either been transferred to WHO headquarters or have been duplicated at headquarters.

13. Future of the Advisory Committee

13.1 The 2011 WHA will determine the future of the smallpox programme and consequently that of the Committee. The WHO Secretariat commented on the method of work of the Committee and requested consideration of its internal functions and how it operates.

COMMITTEE DISCUSSION: The Chair noted that a focus on research with live variola virus has been the remit of the Committee. Other views were that the Committee has succeeded in its task of advising on a programme of research, without focusing on security and logistics. It was also noted that there has been a tendency to focus on regulatory issues, despite a current lack of regulatory expertise within the Committee.

- 13.2 The WHO Secretariat expressed its sincere appreciation for the work of the Committee, commending its work in delivering scientific analysis and oversight. The WHO Secretariat also expressed its sincere appreciation to the repositories for their work. It was noted that this work represents an excellent example of science and policy working together in an effective way.

Annex 1. Summary of presentations

Update on research proposals submitted to WHO

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

25 November 2009: proposals submitted by Vector

1. The maintaining of the Russian national collection of variola viruses
2. The use of live variola virus to provide the development of low reactogenic vaccines

The reviewers recognized the need for simultaneous testing of the same sera for neutralization against vaccinia (as proposed in the project) to obtain data that will confirm or not the requirement for future sero-neutralization studies using variola. The reviewers stressed the importance of including international serum standards in their assays. One reviewer, unfavorable to this project, felt that the ability to neutralize vaccinia has been used extensively in the past to evaluate neutralization activity in serum samples from individuals vaccinated against smallpox and saw no reason to use variola virus instead. This reviewer also pointed out that there is no evidence that an assay for variola virus neutralization is a better correlation of protection against smallpox than an assay for vaccinia virus neutralization.

30 May 2010: proposals submitted by CDC

1. The use of live variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support
2. Use of live variola to evaluate antivirals against variola
3. Use of live variola to develop protein based diagnostic and detection assays specific for variola

One reviewer felt that the project had not sufficiently clarified whether new monoclonal antibodies would be raised against variola or whether the work only involved screening of already existing collections of monoclonal antibodies. The subcommittee recommended approval of this proposal on the condition that this point is clarified.

4. The use of live variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines

Several reviewers noted that insufficient reporting on research into this topic had been provided to the Advisory Committee and called for increased transparency in the form of detailed progress reports.

8 July 2010: proposal submitted by Vector

1. Discovery of antivirals for smallpox treatment and prevention

The reviewers made some suggestions and one request:

- The candidate antivirals should be compared to the most effective smallpox antivirals that have been identified to date, namely ST-246[®] and CMX001. Emphasis should be put on new compounds that have viral targets distinct from those previously found.
- Some of the 90 compounds to be tested could be discarded on the basis of their known or likely toxicity in humans.
- The subcommittee, and in due course the entire committee, should be informed of progress in testing the activity of the compounds in vitro against variola before testing of the compounds in non-human primates with live variola virus, is undertaken.

23 August 2010: proposal submitted by La Trobe University, Australia

1. Discovering the molecular mechanism underlying variola virus inhibition of cell death

The majority of the reviewers felt that this research should not require any approval by the subcommittee as the work does not in any way involve the use of live variola virus (or any other related orthopoxvirus). One reviewer expressed concern about the attention that publication of such work may attract together with a misunderstanding among the public of its consequences. The reviewer recommended that the proposal be reviewed by the committee as a whole before approval.

Update on variola virus clones held at the National Institute for Virology, South Africa

Robert Swanepoel

National Institute for Virology, South Africa

In 1979, the World Health Organization certified that smallpox had been eradicated worldwide, and on 8 May 1980 the 33rd World Health Assembly issued a formal declaration to this effect. Member States were advised to transfer their smallpox virus stocks to one of the two WHO-designated repositories, one in the United States of America, the other in the Soviet Union (now the Russian Federation). South Africa, which had earlier been expelled from WHO, continued to hold smallpox virus stocks at the National Institute for Virology (NIV).

On 9 December 1983 the South African Minister of Health presided over the ceremonial autoclaving and subsequent incineration of the smallpox virus stocks at NIV. The Department of Health had agreed to destroy the stocks of smallpox virus on condition that clones (fragments) of smallpox virus DNA (genetic material), constituting most of the genome of the virus, which had been prepared in the UK for WHO, would be made available for storage at NIV. These clones could theoretically be used to prepare diagnostic reagents, but could not be re-assembled into whole virus.

The clones have never been used and as at October 2010 remain in storage inside the BSL4 of the National Institute for Communicable Diseases (NICD), the successor to NIV. The Department of Health has now decided to retain only those clones that are potentially useful in producing diagnostic reagents, constituting no more than 20% of the genome of the virus. The remainder will either be transferred to the repository at the Centers for Disease Control and Prevention in Atlanta, United States of America, or, in the case of duplicates, destroyed under the supervision of WHO.

"Scientific review of variola virus research, 1999–2010"

Chapter 1 Smallpox vaccines

Antonio Alcami¹ and Bernard Moss²

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Public health importance

Smallpox is the only human disease that has been eradicated by a global vaccination campaign. This accomplishment remains one of the great triumphs of medical science. The smallpox vaccine, which consists of live vaccinia virus, was highly effective. However, it has a history of severe complications, particularly in individuals with an immunodeficiency or with eczema. As well, since it was made in live animals under non-sterile conditions, it would not meet current manufacturing guidelines. There is therefore a clear public health interest in developing a new, efficacious and safe vaccine.

Progress to date

Smallpox vaccines made in tissue culture cells have been produced and licensed. However, these vaccines are likely to induce a rate of adverse effects similar to the original vaccines. Consequently, several approaches have been taken to produce safer vaccines. Progress has been greatest with strains of vaccinia virus that are more attenuated – namely, modified vaccinia virus Ankara (MVA) and LC16m8, which have been produced by repeated tissue culture passage. MVA is more highly attenuated than LC16m8; it has usually been given intramuscularly or subcutaneously, and so does not produce the typical skin lesion that provides evidence of a “take”. LC16m8 can be administered by skin scratch, like the conventional smallpox vaccine, but produces a milder take than the parental virus, vaccinia virus strain Lister. MVA and LC16m8 have been shown in non-human primates to be safe and to produce good immunogenicity, including protection against monkeypox virus, a close relative of variola. New generation vaccines consisting of live vaccinia virus with specific gene mutations, DNA encoding poxvirus genes, and purified proteins have all shown promise in animal models, but none have reached clinical testing.

Outcomes and implications

Licensing of smallpox vaccines grown in tissue culture has been a useful step forward; however, use of these vaccines would be medically contraindicated for individuals with immunodeficiency and certain dermatological conditions. Since smallpox has been eradicated, the efficacy of new generation vaccines will need to be tested using poxviruses related to variola virus in animal protection studies, and safety and immunogenicity studies in humans. However, confidence in the ability of these vaccines to protect against smallpox would be increased by use of live variola virus for in vitro neutralization tests and non-human primate studies.

"Scientific review of variola virus research, 1999–2010"

Chapter 2 Laboratory diagnostics

Inger Damon¹, Hermann Meyer², and Sergei Shchelkunov³

¹ Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, United States of America

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³ Department of Genomic Research, State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, the Russian Federation

Public health importance

Variola virus is the causative agent of smallpox, a disease that was declared eradicated by the World Health Assembly in 1980. The virus is considered a potential biowarfare agent or terrorist weapon due to the high morbidity and mortality it can cause, and because much of the human population is now susceptible due to routine smallpox vaccination being largely discontinued in the 1970s (Henderson et al., 1999)². Taking into account the serious consequences of a smallpox diagnosis or even the consequences of a misdiagnosis, there is a need to be able to identify smallpox unambiguously, rapidly and reliably. This includes an equally reliable differentiation from other similar clinical entities. The predictive value of a positive diagnostic result (also referred to as predictive value positive) is exceedingly low in a low-prevalence disease; diagnostic strategies that improve predictive value positive need to be used.

Progress to date

Between 2000 and 2010, there have been remarkable advances in the clinical and laboratory diagnostic capacities for smallpox. This chapter reviews historical methods for smallpox diagnosis, and summarizes the advances in nucleic acid diagnostic assays, serological assays and protein detection assays developed for smallpox since 2000. Newer technologies have driven the approaches taken by many investigators. Specifically, nucleic acid detection strategies are increasingly using high-throughput real-time polymerase chain reaction technologies and, in some cases, array platforms.

Outcomes and implications

Many nucleic acid-based assays have been developed, but only a few immunology or protein-based diagnostic techniques. All smallpox and poxvirus assays, including these new assays, are research based; none have completed regulatory review and approval processes. The possible need for live variola virus for regulatory review of assays is being discussed at the time of writing. One nucleic acid-based diagnostic kit is available commercially; however, it is for research purposes only and not diagnostic use.

² Henderson DA et al. (1999). Smallpox as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *Journal of the American Medical Association*, 281:2127–2137.

"Scientific review of variola virus research, 1999–2010"

Chapter 3 Variola genomics

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Public health importance

New technologies have radically improved our understanding of the genomics of variola virus. This has led to new ways of detecting and diagnosing smallpox, and insight into the evolutionary history of smallpox infections and the reasons for their severity. However, new technologies in synthetic biology have also created unanticipated problems for controlling access to variola genetic materials. This chapter provides an overview of the latest discoveries in variola virus genomics, and discusses how new technologies in genome synthesis could confound existing strategies for containment of the virus.

Progress to date

The complete DNA sequence of two closely related variola virus genomes was first published in the early 1990s. As a result of an intensified smallpox research agenda, which was approved by the World Health Organization (WHO) Secretariat and begun in 2000, near-complete genome information is now publicly available for 48 geographically distinct isolates of variola virus. These data can be used to improve understanding of variola virus evolution, to develop improved diagnostics, and (with biostructural studies) to provide insights into drug target sensitivities. Working with cloned variola virus genes, researchers have also increased their understanding of interactions and activities of individual variola virus proteins. This provides further important insights into how the virus causes disease in humans.

This chapter summarizes the available genomic information for variola virus, and shows how it has been applied to study the relatedness of the virus to other animal poxviruses, to study virus evolution during human epidemics and to develop diagnostic tests. The chapter discusses the future use of variola virus genomic material in light of new synthetic DNA technologies.

Outcomes and implications

Publicly available genomic information has been used by many international scientists to design highly sensitive virus diagnostics. New information about the relationship between variola virus and other orthopoxviruses is also important for understanding the value and limitations of animal models for human smallpox. As a result of the remarkable expansion in the technologies of DNA synthesis, sequencing and cloning, it is now technically possible to synthesize the entire variola virus genome from scratch, using only publicly available sequence information, and to reconstitute infectious virus using currently available techniques of molecular biology. Future biodefence strategies need to incorporate new thinking about how best to control the application of these synthetic biology technologies.

"Scientific review of variola virus research, 1999–2010"

Chapter 4 The status of WHO-CC repositories

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This chapter summarizes the status (as of January 2010) of live variola virus (VARV) stocks and VARV DNA stocks, and – where appropriate – use and distribution of VARV gene fragments, as per World Health Organization (WHO) recommendations.

In 1976, as efforts to eradicate smallpox met with increased success, the WHO Smallpox Eradication Unit initiated attempts to reduce the number of VARV stocks held in laboratories. As a result, the number of laboratories self-reporting VARV stocks to the Global Commission for Smallpox Eradication decreased from 75 to 7 by December 1979, and subsequently to 4 by 1981. The remaining stocks were located in the USSR, South Africa, the United Kingdom and the United States of America.

In 1982, VARV stocks from Porton Down in the United Kingdom were transferred to the United States, to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. The virus stocks in South Africa, which were maintained at the National Institute for Virology in Sandringham, were destroyed in 1983 (although South Africa still retains cloned, non-infectious VARV fragments).

In May 1996, resolution WHA 33.4 of the World Health Assembly endorsed recommendations for the post-smallpox eradication era. The resolution specified that the remaining stock of VARV should be held at a limited number of sites. The stock has since been reduced, and is currently restricted to two laboratories: the WHO Collaborating Centre on Smallpox and other Poxvirus Infections at the CDC, and the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA at the Russian State Research Centre of Virology and Biotechnology (SRC VB VECTOR) in Koltsovo, Novosibirsk Region, the Russian Federation.

Annual reports from these two laboratories are submitted to the WHO Secretariat. The reports cover use of live VARV and the status of the repositories. Since 2000, these reports have also been made in person at the annual meetings of the WHO Advisory Committee on Variola Virus Research, which are convened to review work with live VARV. Abstracts of these presentations are available online, via the WHO web site.³

³ <http://www.who.int/csr/disease/smallpox/research/en/index.html>

"Scientific review of variola virus research, 1999–2010"

Chapter 5 Animal models and pathogenesis

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Public health importance

The potential for variola virus to be exploited as a bioterrorist weapon is widely understood. In addition, the re-emergence of monkeypox as a public health concern in the Democratic Republic of the Congo has increased the urgency of developing improved countermeasures, including vaccines and antiviral drugs, for these orthopoxviruses. Since it is generally recognized that animal models will be needed to demonstrate efficacy of these countermeasures, this chapter focuses on useful animal models for orthopoxvirus disease.

Progress to date

Small animal models using ectromelia virus (the cause of mousepox), cowpox virus, rabbitpox virus and vaccinia virus have provided insight into the pathogenesis and immunology of poxvirus infections; this knowledge has been used to design critical studies using primates. Primate models using variola virus or monkeypox viruses are the most relevant to the development of safe and effective countermeasures against smallpox in humans.

Current challenges

Various combinations of variola virus doses and routes of exposure in primates (cynomolgus monkeys) lead to predictable disease patterns that replicate some, but not all, features of human smallpox. Although the models require further refinement, they have been adequate to demonstrate the efficacy of several candidate antiviral drugs, including cidofovir and ST-246®. It is likely that no single combination of conditions will result in a model that will simultaneously satisfy all of the criteria required under the United States Food and Drug Administration 'Animal Rule' (US 21CFR310.610); different models may be required to assess different indications. Further refinement of the primate models might include pathophysiological data from studies using telemetry and medical imaging. Special attention should also be paid to finding biomarker patterns that could be used in a clinical setting as triggers for early intervention, thus increasing the likelihood of successful intervention and facilitating the licensing of countermeasures. Although much of this developmental work can be accomplished using surrogate orthopoxvirus in rodents and primates, increased confidence in countermeasures against variola virus can be obtained only by efficacy testing in primate models using variola virus.

"Scientific review of variola virus research, 1999–2010" Chapter 6 Antiviral drug development for smallpox treatment

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Public health importance

Widespread vaccination against smallpox is extremely unlikely to occur before the first occurrence of a smallpox outbreak, because of the serious and occasionally fatal events associated with current smallpox vaccines. Therefore, if smallpox re-emerges, it may be necessary to treat a large number of cases with an antiviral drug before mass vaccination campaigns have time to provide adequate protective immunity.

Previous smallpox control measures have had to rely exclusively on vaccination and supportive care of infected individuals, who may be facing a 30% chance of dying from the infection. However, experience with control of the current H1N1 influenza epidemic has shown that both vaccine and antiviral drugs can be important as part of the public health response, both to control the outbreak and to reduce mortality in those infected.

The project described in this chapter was undertaken to obtain two approved oral antiviral drugs, with different mechanisms of action, for treating clinical cases of smallpox. These drugs need to have been approved by drug regulatory agencies if they are to be used during an outbreak. Regulatory approval also provides convincing evidence of the efficacy of the drugs, which will be needed by public health officials who are formulating control strategies. Because smallpox, caused by variola virus, was eradicated by mass vaccination, the effectiveness of these drugs can only be demonstrated using variola virus-infected animal models in non-human primates.

Progress to date

Development of any antiviral therapeutic is a long and difficult process, which has been unsuccessful for many viral infections, including the common cold. For smallpox, considerable progress has been made in initial drug discovery, and a number of potential candidates need to be evaluated in animal models.

Three compounds – cidofovir, ST-246[®] and CMX001 – that inhibit variola virus replication, in cell culture and in multiple animal models (surrogate orthopoxvirus models), have gained investigational new drug (IND) status from the United States Food and Drug Administration (FDA) for treatment of orthopoxvirus infections. Initial human studies are in progress. Two of these compounds (cidofovir and ST-246[®]) have demonstrated activity in a lethal primate model of variola virus, and the third is a prodrug of cidofovir that can be given orally. Development of ST-246[®] and CMX001 is in progress, and clinical trials are ongoing.

Additional work requiring live variola virus to obtain an approved antiviral drug for the treatment of smallpox

Although results to date are promising, extensive industry experience with drug development suggests that fewer than 35% of compounds entering expanded safety trials (FDA phase II) will obtain approval. The process of moving from the IND stage to the new drug application stages takes an average of five to seven years. Since variola virus has been eradicated from the human population, traditional clinical efficacy trials are not feasible. In addition, it is not possible to conduct ethical clinical trials in humans, so demonstration of efficacy must use the FDA ‘Animal Rule’ (US 21CFR310.610). Given the uncertainties in the ‘Animal Rule’, and the fact that no antiviral drugs are currently approved for any type of smallpox indication (treatment or chemoprophylaxis), it is difficult to estimate the time lines or the data required for approval; the data are expected to include, but not be limited to, work with variola virus. The intensity of review and the level of scientific scrutiny applied to animal model studies proposed to support indications under the ‘Animal Rule’ would be the same as for human clinical trials to support approval of products for other types of indications using other approval pathways.

Approval in countries other than the United States of America is associated with at least as much uncertainty. It could be argued that work with live variola virus must remain an option until an adequate number of drugs, with different mechanisms of action, have gained regulatory approval and could be used worldwide to combat an outbreak of smallpox.

A report by the Institute of Medicine of the National Academies, entitled *Live variola virus considerations for continuing research*, concluded that “the most compelling reason for long-term retention of live variola virus stocks is their essential role in the identification and development of antiviral agents for use in anticipation of a large outbreak of smallpox”.

"The Advisory Group of Independent Experts to review the smallpox research programme: comments on the *Scientific review of variola virus research, 1999–2010*

Tania Sorrell and Rakesh Aggarwal on behalf of the AGIES.

The Advisory Group of Independent Experts (AGIES) found the *Scientific review of variola virus research, 1999–2010* to be clearly written and comprehensive, and to provide an accurate and up-to-date review of variola virus research, including the impact of regulatory restrictions on current and future research.

The full report is presented in three parts, in line with the terms of reference provided to the AGIES.

In Part 1, a summary of each chapter of the Scientific Review is provided, followed by specific comments. Part 2 contains the committee's recommendations for further research and comments on variola virus (VARV) repositories. Part 3 summarizes AGIES recommendations for ensuring high standards of security related to re-emergence of smallpox.

Recommendations for further research and for use of live variola virus

Part 1: Genomics, diagnostics and repositories

Genomics

Near complete genomic sequences are available for approximately 50 isolates of variola virus. Since variola virus genome has only limited genomic diversity and shows major homologies with genomes of other orthopoxviruses, the AGIES feels that there is no public health need for sequencing of additional variola virus isolates.

Diagnostics

Several nucleic acid-based assays have been developed. Some have used cloned or synthetic genetic elements from variola virus DNA for assay evaluation and development, others have used intact genomic variola DNA; some have used cloned variola DNA as positive controls. Their further development does not require the use of live VARV.

In the absence of clinical disease, it is not possible to determine the sensitivity, specificity, positive and negative predictive value of these tests in clinical situations. There is a need for regulatory validation of these assays. Head-to-head comparisons and further optimization of the available assays, especially real-time polymerase chain reaction (PCR) and microarray platforms, should be undertaken. Newer assays should be developed as advances in diagnostic technology are made.

Several serological tests are available to detect antibodies to orthopoxviruses. Antigen capture assays are early in development; to date, are generic for the orthopoxviruses, and none are variola specific. These may also benefit from head-to-head comparison. Efforts to improve their performance characteristics are warranted; newer assays may be developed as advances in diagnostic technology are made.

Live variola virus

The AGIES is of the view that live variola virus is not required for the further development of diagnostic tests nor for technical assay validation.

Repositories

See Part 3. Security issues.

Part 2: Vaccines, animal models and drugs – future research and requirement for use of live variola virus

Vaccines

The AGIES believes that attempts must continue to develop vaccines that are safer than, and at least as efficacious as, the original and/or existing licensed vaccines against smallpox.

In order to prepare for a potential outbreak of smallpox, strategies for efficacious therapeutic immunizations, such as post-exposure vaccination or delivery of anti-vaccinia immunoglobulins and/or monoclonal antibodies, need to be developed. These approaches should help to shorten the response time of public health systems in case of an outbreak. In addition, passive immunization may ameliorate the adverse effects of available vaccines in special subgroups.

Animal models

Neither variola models in animals nor natural poxvirus infection in animals can exactly model human smallpox.

Although current non-human primate models using VARV are suboptimal, research conducted into developing them further over the last decade has achieved limited success. The only reason for attempts to develop such models is to meet the current stringent regulatory requirements, in the absence of human variola virus infection. The AGIES's opinion was that a more productive approach would be for the regulatory requirements for vaccine and drug approval for variola virus infection to be reconsidered, given that human infection with the virus no longer occurs.

Therefore, the AGIES recommends that rather than develop animal models using VARV, research should concentrate on improving surrogate models that use infection with other orthopoxviruses in their natural hosts (e.g. monkeypox virus (MPXV), cowpox virus (CPXV), rabbitpox virus (RPXV), ectromelia/mousepox virus (ECTV), infections in non-human primates, rabbits and rodent models).

Such models would allow studies on pathogenesis of poxvirus infection, the analysis of drug and vaccine efficacy, and the establishment of criteria to evaluate protection.

Drugs

Two anti-variola drugs, namely, cidofovir and ST-246[®], are in advanced stages of development. Resistance to each of these drugs has been described in vitro but the risk of treatment-induced resistance in vivo is not known. The AGIES recommends that if the development of resistance in vivo is deemed by ACVVR to be a significant possibility, then additional drugs with alternative mechanisms of antiviral action should be developed. However, in the first instance, efforts should primarily focus on cidofovir and ST-246[®].

Live variola virus

At present, assuming that regulatory issues around vaccine and drug testing are resolved, the only indication for use of live VARV is to test the efficacy of drugs in vitro.

Part 3: Security issues

Monitoring containment policies

Stringent regular review of the quality assurance and containment practices at the Russian Federation's State Research Centre of Virology and Biotechnology (SRC VB VECTOR) and the U.S. Centers for Disease Control and Prevention (CDC) must be continued.

Genetically engineered VARV, mutants of VARV or poxviruses containing parts of the VARV genome

The AGIES makes the following recommendations.

New strategies should be designed to address the potential for de novo synthesis of live VARV, including adoption of national policies on the issue by WHO Member States. It is recommended that recent biosecurity proposals (Bügl et al. 2007)⁴ be considered at national policy level.

The World Health Organization should seek an updated validation from all countries regarding their stocks of variola virus DNA (in various forms, such as fragments, amplicons and/or plasmids).

With respect to the current prohibition on laboratories, other than the two WHO collaborating laboratories that retain more than 20% of the VARV genome, it is recommended that both CDC and VECTOR provide documentation to WHO cataloguing which segments of DNA have been distributed to which laboratories. Advice should also be sought on whether the complete genome has been distributed (or should be distributed), albeit as different gene segments comprising <20% of the genome, to a range of laboratories.

⁴ Bügl H et al. (2007). DNA synthesis and biological security. Nature Biotechnology, 25:627–629.

The subcommittee for the establishment of a Smallpox Laboratory Network

Jean-Claude Piffaretti

Interlifescience, Massagono, Switzerland

A subcommittee has been convened to discuss the establishment of a global WHO network of high-level diagnostic laboratories, the Smallpox Laboratory Network (SLN), which would have the capacity to rapidly and accurately detect any emergence of smallpox virus. The purpose of the SLN would be to alert WHO as quickly as possible to any serious event related to a possible emergence of smallpox. The subcommittee currently comprises representatives of the two reference laboratories (CDC and VECTOR) and representatives of each WHO Region.

The scope of the SLN is currently being defined. It would comprise the two reference laboratories as well as up to two regional laboratories from each WHO region. The regional laboratories would have the capacity to perform rapid and reliable smallpox virus identification using only molecular DNA-based techniques. The two reference laboratories already perform smallpox viral cultures and have the capacity to undertake related diagnostic methods, including typing of smallpox viral strains. The reference laboratories would perform the final confirmatory diagnosis of smallpox viruses and then store the identified viral strains in their repositories.

A candidate regional laboratory would be required to fulfil a number of criteria related to competence, biosecurity, and biosafety, in order to qualify for inclusion in the network. These criteria would include:

- regular diagnostic activity in medical virology and molecular techniques including Real-Time PCR;
- successful regular participation in proficiency assays including those organized by WHO together with the reference laboratories for orthopox/smallpox viruses;
- controlled access to an enhanced BSL-3 laboratory;
- successful inspection by an ad hoc WHO committee;
- capacity to process samples rapidly within a limited period of time (e.g. within 8 hours from alert);
- pre-vaccinated personnel, etc.

The SLN would be coordinated by a steering committee with representatives from each of the laboratories in the network as well as a representative from WHO Secretariat. The SLN steering committee would:

- organize the activities of the laboratory network;
- establish and approve the procedures to be used by the laboratories, particularly those related to the sample acceptance and inactivation, as well as the diagnostics methods used;
- verify on a regular basis, that the above procedures and methods are updated to reflect advances in current science;
- verify on a regular basis that the regional laboratories are continuing to fulfilling the criteria;
- check the results of the proficiency assays;
- provide support to the regional laboratories as needed.

It is recognized that some Member States may have one or more highly competent laboratories that have been authorized by their government to perform smallpox molecular diagnostics. These laboratories should be notified to the WHO Secretariat and should be authorized to communicate directly with the reference laboratories and the WHO Secretariat.

It has been suggested that the SLN may ultimately be subsumed within a more general diagnostic laboratory network under the IHR (International Health Regulations).

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

Investigators: **Inger Damon, Kevin Karem, Victoria Olson**

WHO Collaborating Centre for Smallpox and other Poxvirus Infections, Atlanta, GA, United States of America

Public health importance

The ability to validate nucleic acid-based and protein-based diagnostic capacity is critical for early detection and recognition of smallpox if reintroduction becomes a reality. The consequences of either false negatives, or false positives, could result in significant delays and mistakes in the public health response to a smallpox outbreak. In light of this, there remains a need to maintain variola DNA and antigen stocks to continue research in compliance with protocols approved by the World Health Organization (WHO).

Over the course of approved research, variola DNA stocks have been depleted. These stocks were used to evaluate various nucleic acid-based diagnostic assays for validation and proficiency exercises with worldwide partners. This non-infectious material, which is representative of what would be extracted from a clinical isolate, is invaluable in validating nucleic acid-based diagnostics. Although plasmids expressing the relevant target portions of DNA can be used as internal assay positive controls, assay validation is substantially more robust when materials as close to the authentic clinical isolate are used. For sensitivity analyses, use of virus DNA that has been extracted from purified virions allows a calculation of the level of detection (LoD). Such materials will continue to be used to validate detection assays, as well as human clinical diagnostics. Furthermore, non-infectious material is also required to test protein-based diagnostic assays in preliminary phase research.

Methods

All work with live variola virus is conducted at Biosafety level 4 (BSL 4) under the Terms of Reference of the WHO Collaborating Centre for Smallpox and other Poxvirus Infections at the Centers for Disease Control and Prevention, in Atlanta, US. The facility is reviewed for safety and biosecurity practices by independent teams from the US and WHO on a frequent basis. Any use of variola DNA or antigen outside of the BSL 4 laboratory requires inactivation of the virus. Variola virus is inactivated prior to nucleic acid extraction by heat and lysis methods. Material for antigen preparation is inactivated by gamma irradiation.

Results

DNA diagnostics

In order to thoroughly evaluate nucleic acid-based diagnostic assays, a complete DNA panel isolated from near neighbours must be assessed to validate specificity. One orthopoxvirus, cowpox virus, has been shown to have extensive phylogenetic diversity – based upon limited sequence analysis. Several newly acquired cowpox virus isolates were found, surprisingly, to cross-react in the real-time PCR assay with a previously validated variola-specific signature.

Protein diagnostics

A hybridoma line of mouse monoclonal antibody has specific reactivity to variola virus. However, the reactivity was found to be highly biased towards gamma irradiated antigen compared to live variola virus antigen. The variation of reactivity was found to be specific to the gamma irradiation inactivation procedure since heat, UV or formalin inactivation methods showed inferior reactivity (similar to live variola virus antigen) compared to gamma irradiated variola virus antigen.

Discussion/Future directions

Despite the fact that the variola-specific target was designed within a highly conserved gene, the region was found not to be unique to variola virus, with a similar sequence also found within the human pathogen cowpox virus. The variola-specific signatures of other published diagnostic assays are currently being compared with bioinformatic data to determine if they retain their specificity based upon these newly acquired cowpox virus sequences. The possibility of a variola virus-specific assay cross-reacting with the near neighbour cowpox virus dramatically increases the chance for false positive results, which may lead to a breakdown of public health infrastructure. Future work will generate greater bioinformatic data by sequencing genomic DNA from other near-neighbour viruses and evaluating new signatures for specificity against a broader DNA panel of variola virus, near neighbour viruses, and other rash-causing pathogens. Together, this data will assist in providing the most specific and sensitive real-time PCR assay possible for the reliable detection of variola virus.

With regards to protein-based research, studies have been initiated to “pan” for antigen epitopes in the hope of finding the epitope recognized by the variola-specific monoclonal antibody. Another screening project has begun to evaluate frozen hybridomas (previously generated) for new monoclonal antibody candidates. Experiments continue to generate a protein bank of variola proteins for use in protein microarray analysis. Reagents resulting from these studies will ultimately benefit from confirmation of findings with live variola virus. These continuing efforts will provide options regarding protein-based viral detection as well as serologic tests to enhance orthopoxvirus testing.

Update on the current status of the collection of variola virus strains and discovery of antivirals against variola

Evgeny Stavskiy and Sergei Shchelkunov

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

WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA, Koltsovo, Novosibirsk region, Russia

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

- freeze-dried and frozen cultures – 120 strains;
- 17 primary specimens isolated from human patients in the past;
- total number of registered stored units – 691.

In 2010, work with variola virus continued in the following areas:

- glass vials containing viruses held under frozen conditions were replaced by polypropylene cryovials with printed labels resistant to disinfectant solutions. This was done in order to improve safety during both storage and work;
- testing of the antiviral properties of compounds with previously identified antiviral efficacy against other orthopoxviruses;
- testing of the neutralizing properties of mini-antibodies with previously identified neutralizing activity against other orthopoxviruses.

All VARV cultures that were previously stored in glass vials have now been transferred into polypropylene cryovials.

Approximately 90 chemical compounds of different classes (heterocyclic derivatives, nucleoside derivatives, adamantane derivatives, etc.) were studied. The most promising of these chemical compounds passed a full cycle of testing on surrogate viruses *in vitro* and *in vivo* (vaccinia, cowpox and ectromelia). Based on the results of these tests, the 31 most promising chemical compounds were selected for further research *in vitro* to assess the presence of antiviral activity against four variola virus strains with differing levels of virulence (6-58, Ind-3a, Congo-9, and Butler). The greatest anti-smallpox activity among these compounds was demonstrated by four compounds synthesized by the Novosibirsk Institute of Organic Chemistry at the Siberian Branch of the Russian Academy of Sciences.

A panel of both previously and newly synthesized single-chain human antibodies against orthopoxviruses was tested for their ability to inhibit the infectivity of variola virus. The presence of virus-neutralizing properties was tested in plaque-reduction neutralization tests of variola virus in eukaryotic cell culture Vero. Murine monoclonal antibodies 2D5 were used as a positive control. The study was carried out at a steady titre (250 PFU/ml) of variola virus, strain Ind-3a. The findings demonstrated that four out of seven single-chain human antibodies studied were capable of neutralizing variola virus infectivity.

The collection of variola virus is now permanently stored in the newly established repository in the WHO CC facility designated for research on VARV. This repository is equipped with a complete set of physical security systems as well as with warning and fire alarms.

The use of live variola virus to evaluate antivirals against variola

Inger Damon, Kevin Karem, Victoria Olson

WHO Collaborating Centre for Smallpox and other Poxvirus Infections, Atlanta, GA, United States of America

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

Public health importance

The primary objective of preparedness for smallpox bioterrorism is to save lives if smallpox somehow reemerges. The availability of therapeutics for smallpox would provide significant advantages during an outbreak, enabling treatment to be administered after exposure. A study by Stittelaar *et al.* published in *Nature*, on 11 December 2005 described a lethal intratracheal monkeypox infection and demonstrated that treatment with an antiviral compound at the time of infection was protective, whereas vaccination was not. The results appear to challenge the limited data, gathered during smallpox eradication, on the efficacy of vaccination up to 4 days post-exposure to prevent smallpox. Contemporary opinion has placed the need at two antiviral compounds, with discrete mechanisms of action, to be licensed and available for use. Compounds specifically targeting viral proteins, viral processes, or cellular functions required by the virus but non-essential for the human host, are desired. Evaluation of therapeutics requires *in vitro* and/or animal model characterization of their activity against live variola virus infection. To date, several agents have shown promise in effecting host cellular targets to inhibit viral replication, or direct targeting of viral proteins required for viral replication and maturation. The successful development of these agents requires testing against live variola virus to evaluate and ensure direct effect and efficacy of these compounds.

Methods

All work with live variola virus is conducted at Biosafety level 4 (BSL 4) under the Terms of Reference of the WHO Collaborating Centre for Smallpox and other Poxvirus Infections at the Centers for Disease Control and Prevention, in Atlanta, US. The facility is reviewed for safety and biosecurity practices by independent teams from the US and WHO on a frequent basis.

Results

Evaluation of tyrosine kinase inhibitors

Previous work with orthopoxvirus vaccinia has suggested that certain families of cellular tyrosine kinases are involved in viral egress from the infected cell. Several compounds inhibit one or both families of cellular tyrosine kinases (Abl- and Src- family), which are currently used therapeutically for treatment of chronic myelogenous leukemia and stromal tumors. Quantitation of extracellular enveloped virus (EEV) and mature virion (MV) production in the presence of tyrosine kinase inhibitors confirmed they specifically inhibit production/release of EEV. Understanding the mechanism of action of these potential therapeutics is critical, not only for patient safety, but also for possible design of future therapeutics. Both variola and monkeypox viruses egress from cells via actin tails, as seen with vaccinia virus. Furthermore, the licensed drugs and their derivatives prevented EEV egress of monkeypox virus and variola virus. Cellular tyrosine kinases are necessary for efficient release and spread

of EEV during orthopoxviral infection; Src-family tyrosine kinases are required for formation of actin tails while Abl-family tyrosine kinases function in release of EEV. Our data supports these cellular tyrosine kinases as viable targets for therapeutics and has recently been accepted for publication in the *Journal of Virology*. Since these therapeutics target cellular proteins, this approach is unlikely to engender viral resistance.

Evaluation of a herbal remedy for smallpox

Research within Jeffrey Langland's laboratory has recently "rediscovered" a carnivorous plant as having anti-orthopoxvirus activity. Multiple historic reports exist on the successful treatment of smallpox outbreaks in the North American continent with this botanical extract. Langland's group has demonstrated the extract effectively inhibits viral replication and the viral-induced cytopathic effects of various orthopoxviruses. At doses where virus replication was inhibited, little to no cellular toxicity was observed. Viral replication was blocked initially but partial replication was observed soon after, likely due to breakdown or utilization of active components within the extract. However, treating the cells with fresh extract every six hours completely abolished viral replication. The extract effectively inhibits the replication of vaccinia, monkeypox, and variola viruses acting at the stage of early viral transcription. The inhibitory effect was specific to orthopoxviruses, and did not significantly affect the replication of other viruses tested. Finally, other herbal remedies tested did not influence vaccinia virus replication. This activity towards orthopoxviruses indicates its potential as a therapeutic agent.

Discussion/Future directions

Future work will focus on further characterization of the extract: identification of the active component (which may necessitate further anti-variola evaluation), and efficacy within an animal model of systemic orthopoxvirus disease.

There are other interesting potential anti-variola compounds to evaluate; at least two new promising classes (5-substituted 4'-thiodeoxyribonucleosides, proteasome inhibitors) of antivirals have shown promise against other orthopoxviruses, such as vaccinia and cowpox viruses. The use of live variola virus to determine the efficacy of these compounds in vitro has the potential to identify anti-viral agents with unique mechanisms of action at different stages of the viral life cycle.

Perspectives on the development of primate models for evaluating countermeasures against human smallpox and monkeypox

Peter Jahrling

Integrated Research Facility, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Frederick, United States of America.

Since 1999, some progress has been made in developing animal models, however it should be emphasized that there is still no animal model that satisfactorily recapitulates all relevant aspects of human smallpox. Variola virus can be useful for understanding human physiology and immunology because it has the capacity to overwhelm the host in a way that few viral pathogens do. Further research is needed to develop improved animal models that can recapitulate key aspects of the human disease and to understand virus–cell interactions in human target cells relevant to pathogenesis and immune response.

New studies on the pathology of human smallpox in comparison with cynomolgus macaques challenged intravenously with variola and monkeypox viruses have shown that the role of concurrent infections, the mechanisms of lymphoid necrosis and lymphoid hyperplasia, the reproductive and lung and kidney pathology need further investigation.

While the need for parallel studies with monkeypox is critical and significant progress has been made with variola models, more work remains. Models should be optimized by varying viral strain, dose and route of exposure to attain specific objectives. Studies should continue to determine mechanisms of lymphoid necrosis, hyperplasia, and apoptosis, to improve analysis of coagulopathy/fibrinolytic processes and to identify relevant biomarkers for clinical intervention.

Update on ST-246[®] development

Dennis E Hruby

SIGA Technologies, Inc., United States of America

There is a need for effective inhibitors for poxvirus-induced diseases, such as smallpox caused by variola, which is a potential biological warfare agent. Likewise emerging zoonotic infections due to cowpox virus and monkeypox virus require the development of effective countermeasures.

SIGA's smallpox antiviral ST-246[®] meets this unmet need and is in late stage development and procurement-ready. ST-246[®] has shown efficacy in all small animal and non-human primate efficacy models tested to date.

Phase II clinical trials have been completed along with the NDA-enabling toxicology studies. SIGA is in the midst of commercial manufacturing and preparation for the pivotal safety and efficacy studies. SIGA's 2010 update will focus on the completion of scale-up manufacturing preparations, progress towards regulatory approval, and ongoing efforts to satisfy the 'Animal Rule' using both monkeypox and variola virus non-human primate models.

Status of CMX001 development for smallpox and other dsDNA viruses

Randall Lanier, Scott Foster, Bernhard Lampert, Tim Tippin, Lawrence C. Trost, Rose O'Mahony, Laurie Keilholz, Alice Robertson, Merrick Almond and George Painter.

Chimerix Inc., Durham NC, United States of America

CMX001 is a lipid conjugate of the acyclic nucleotide phosphonate, cidofovir (CDV, Vistide®). Both CMX001 and CDV inhibit viral DNA replication and are active in vitro against all 5 families of double-stranded DNA (dsDNA) viruses that cause human morbidity and mortality, including orthopoxviruses such as variola. However, the clinical utility of CDV is limited by the need for intravenous infusion and a high incidence of acute kidney toxicity.

CMX001 is currently in Phase II clinical trials for prophylaxis of human cytomegalovirus infection and under development using the FDA's 'Animal Rule' for smallpox infection. It has proved effective in reduction of morbidity and mortality in animal models of smallpox, even after the onset of clinical signs of disease, including lesions. CMX001 has a number of advantages over CDV and other drugs in development for treatment of smallpox, including broad spectrum inhibition of dsDNA viruses that cause human disease, a high genetic barrier to resistance, convenient oral administration as a tablet or liquid, and no evidence to date of renal toxicity.

The absence of nephrotoxicity is supported by in vitro data demonstrating that CMX001 is not a substrate for the human organic anion transporters that actively secrete CDV into kidney cells. CMX001's resistance profile and the ability to test the safety and efficacy of CMX001 in patients with life-threatening dsDNA virus infections which share many basic traits with variola virus are major advantages in the development of this antiviral for a smallpox indication.

Clinical development status of the third generation non-replicating smallpox vaccine IMVAMUNE®

Lars Staal Wegner

Bavarian Nordic, Kvistgaard, Denmark

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

More than 3200 subjects have been vaccinated with > 5000 doses of IMVAMUNE®, including more than 1000 subjects from risk groups with contraindications for conventional smallpox vaccines, i.e. HIV infected and atopic dermatitis patients.

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

IMVAMUNE® development is supported by US Government contracts DMID N01-AI-30016, DMID N01-AI-40072, HHSO100200700034C and in May 2010 the first 2 million doses of a total of 20 million was delivered to the US Strategic National Stockpile, under Emergency Use Authorization (EUA).

Update on the use of smallpox vaccine LC16m8 in Japan

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² National Institute of Infectious Diseases, Toyama Shinjuku-ku, Japan

Background

Prior to the 1970s, conventional smallpox vaccines were known for their efficacy while causing a number of adverse events (AEs). In 1972, the Japanese Ministry of Health and Welfare established the Smallpox Vaccine Research Committee (SVRC) to conduct research into smallpox vaccination-related AEs. As part of this research, over 10 000 children were vaccinated with LC16m8, resulting in milder AEs compared to those previously observed with other conventional smallpox vaccine strains. Consequently, the LC16m8 vaccine was licensed in 1975 in Japan.

In light of increasing global concerns about possible bioterrorism with smallpox (variola) virus, Kaketsuken resumed the manufacturing of LC16m8 vaccine for a national stockpile upon request of the Japanese government. In addition, a new research structure, Smallpox Vaccine Research Group (SVRG), consisting chiefly of the National Institute of Infectious Diseases (NIID), Kaketsuken and Keio University, was established to conduct basic and clinical research on LC16m8.

Clinical research

Children

A total of 9538 subjects were vaccinated with LC16m8. Their health conditions were observed and daily body temperature was taken for 1 month post-vaccination. The results are as follows:

- "Take" (a major skin reaction) rate: 9075 subjects (95.2%)
- fever: 663 (7.0%), eczema vaccinatum: 1 (0.01%)
- auto inoculation: 9 (0.09%)
- satellite vesiculation: 28 (0.29%)
- post-vaccinal exanthem: 8 (0.08%)
- temporary benign febrile convulsions: 3 (0.03%).

Adults

A total of 3221 adult subjects (including 1529 vaccinia-naïve adults) from the Japan Self Defense Forces were vaccinated with LC16m8. The "take" rates at 10–14 days after vaccination were 94.4% for vaccinia-naïve adults and 86.6% for vaccinia-experienced adults. Seroconversion or an effective booster response with "take" at 30 days post-vaccination were observed in 90.2% for vaccinia-naïve adults and 60.0% for vaccinia-experienced adults. One case of allergic dermatitis and another of erythema multiforme were noted as suspicious vaccination related reactions. Both cases were mild and rapidly recovered. No serious AE was observed.

Post marketing surveillance (PMS) study

A PMS study was conducted in Japan with 268 healthy adults (196 vaccinia-naïve and 71 vaccinia-experienced). Neutralizing antibody titre was measured for 100 subjects from

vaccinia-naïve and vaccinia-experienced adults. There was no report of serious AEs or death caused by the vaccination. Adverse reactions were observed in 58 individuals, a frequency of 21.6% of adverse reactions. Major adverse reactions included lymphadenopathy (19.4% frequency), erythema at the injection site (5.2%), and fever (1.5%). Meanwhile, “take” rate was evaluated with results from all 268 individuals and was found to be 94.4%.

Use in immunocompromised subjects

Adults

Two subjects showed signs of possible severe AEs caused by vaccination. A 26 year-old vaccinia-naïve male experienced rash onset on the third day post-vaccination; the rash spread from the extremities to the trunk. He was hospitalized at 20 days post-vaccination. The case was diagnosed to be allergic dermatitis by skin biopsy of the rash. A 29 year-old vaccinia-naïve male experienced rash onset at 10 days post-vaccination in the trunk and was diagnosed to be erythema multiforme.

Subjects with an allergic history

An enlarged lymph node was observed in one subject with an allergic history to pyrazolone drugs, which disappeared within a month. No AE was observed in subjects with eczema.

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

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

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Additional external collaborators:

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Frances Newman, Sharon Frey, Robert Belshe, St. Louis University
Bernard Moss, LVD/NIAID/NIH

Supported by: DMID/NIAID/NIH

Public Health Importance

Smallpox vaccines, comprising live, fully replicative strains of vaccinia virus, were successfully used as the primary public health intervention to eradicate smallpox. However, an unacceptable frequency of adverse events (AEs), some life threatening, occurred. The increase in immunosuppressive conditions worldwide now makes it likely that widespread use of these vaccines as the sole public health intervention would have the consequence of a higher degree of undesirable AEs. Hence, there is a need for a new smallpox vaccine with fewer side effects – one that is safe and efficacious. In the absence of an animal model utilizing variola virus, the only direct demonstration of the effectiveness of the elicited immune response against variola virus is plaque reduction neutralization test (PRNT) of infectivity *in vitro*. The true efficacy of reduced reactogenic vaccines, also known as “third” generation vaccines, to prevent smallpox is unclear. The role of variola virus neutralization as a marker for vaccine efficacy is perhaps greatest for the evaluation of these vaccines that do not elicit a “take”, the traditional measure of vaccine success.

Methods

All work with live variola virus is conducted within the maximum containment laboratory at Biosafety level 4 (BSL 4) under the Terms of Reference of the WHO Collaborating Centre for Smallpox and other Poxvirus Infections at the Centers for Disease Control and Prevention, in Atlanta, US. The facility is reviewed for safety and biosecurity practices by independent teams from the US and WHO on a frequent basis.

Results

To determine how well the neutralization tests agree, Geometric Mean Titres (GMTs) were calculated for both the 60% and 90% neutralization titres for each orthopoxvirus neutralization target used by the two centres (SLU [vaccinia virus -Dryvax and MVA] and CDC [variola virus]). Although no significant differences were noted when the 60% neutralization titres were compared, a more robust comparison was gained from the 90% neutralization titres. Using paired t-test, statistically significant differences were noted between the different antigen targets. Spearman correlation coefficients found weak positive correlations for each target comparison. Viral preparations contained similar ratios of

genomes per plaque-forming unit, discounting the possibility that differences in quality of viral preparations may account for the differences in PRNTs.

Individual variability was noted in the overall kinetics of the anti-variola immune response measured by variola virus-Solaimen neutralization. The majority of participants showed a boost in 50% anti-variola virus PRNT between dose one and two of MVA. Although a slight decline in 50% variola virus PRNT was observed by 6 months post enrollment in the trial, all those “challenged” with standard Dryvax vaccination showed an amnestic boost in anti-variola virus-Solaimen 50% PRNT titres when sera were evaluated ~30 days later. Similar rates of 4-fold increase rise in titre were seen for both MVA vaccination regimens. When aggregate data is evaluated, generally equivalent 50% variola virus-Solaimen PRNT geometric mean titres (GMT) was observed two weeks post either MVA regimen. Neutralization capacity boosted one month post “challenge” with Dryvax for both vaccination regimens. At one year post vaccination, while GMTs declined, those boosted by Dryvax had a higher GMT than those who were not challenged regardless of administration route.

Discussion/Future directions

Historically, the “take” was used as a correlate of successful vaccination/inferred protection. New “third generation” smallpox vaccines do not induce a noticeable dermatological response. The reanalysis of the data from DMID 02-017 suggests that significant differences in endpoint titres are observed using different viruses as the substrate for neutralization. Using vaccinia virus-Dryvax as the neutralization antigen underestimates, while using vaccinia virus-MVA as the neutralization antigen would overestimate the variola virus-Solaimen neutralization titre. Perhaps more troubling is the apparent lack of linearity between individuals’ responses. Our analysis here, in combination with data from other vaccine trials, may assist in determining if neutralization titres with vaccinia virus as the target can be bridged to variola virus neutralizing titres. Furthermore, understanding the immune response post MVA vaccination regimen is critical in identifying a correlate of protection.

Baden et al. tentatively identified a correlation between highest MVA-PRNT titre after second dose of MVA and subsequent attenuation of “take” and duration of viral shedding after Dryvax “challenge”⁵. We plan to evaluate if this correlation also exists with variola virus neutralizing titres. To achieve this, we will need to evaluate remaining participants’ sera since the numbers of participants in the two groups already tested do not provide enough statistical power. These data will also further our ability to compare the anti-variola immune response administered via intradermal vs. subcutaneous route. Preliminary observations suggest the intradermal route of administration may be vaccine virus dose-sparing. Additionally, these data will allow us to more fully characterize the kinetics of the anti-variola response.

⁵ Seaman MS et al. Effect of vaccination with modified vaccinia Ankara (ACAM3000) on subsequent challenge with Dryvax. *Journal of Infectious Diseases*. 2010, 201:1353-1360.

WHO smallpox vaccines stocks, status update

World Health Organization, Smallpox Secretariat

WHA resolutions:

In its final report of 1979, the Global Commission for the Certification of Smallpox Eradication discussed the need to maintain reserve stocks of vaccine and concluded that it would be prudent for WHO and national authorities to be prepared for unforeseen circumstances. The Commission recommended that freeze-dried smallpox vaccine sufficient to vaccinate 200 million people should be maintained by WHO, together with stocks of bifurcated needles. In 1986, the global reserve was gradually reduced.

In 2004, the Ad Hoc Committee on Orthopoxvirus Infections recommended that:

- WHO create, monitor and maintain a strategic stock of smallpox vaccines to be held in Geneva. This strategic stockpile should be made available for emergency use only. The volume of the Geneva stockpile should be at least 5 million doses.
- a stockpile of smallpox vaccine should be created through pledges made by Member States. The size of the pledged stockpile should be at least equivalent to the amount that was available to WHO at the end of the eradication programme (200 million doses).

In 2004, the Ad Hoc Committee on Orthopoxvirus Infections recommendations were noted and approved by the World Health Assembly in 2005.

Vaccine stockpile

WHO has built a strategic stock of smallpox vaccines of 30.5 million doses, stored in Switzerland. Ninety-eight percent (30 millions doses) of the WHO strategic stock of smallpox vaccines is second generation vaccine. Two percent (530 000 doses from Belgium, Germany, the Netherlands, Iran (Islamic Republic of), and the Russian Federation) of the WHO strategic stockpile is first generation vaccine.

In addition, through a virtual stockpile mechanism, four Member States have pledged a further 27 million doses to WHO in case of additional needs: France, Germany, New Zealand, USA. WHO has agreed Standard Operating Procedures with all four Member States.

As of 1 July 2010, WHO has established stocks totalling 57.5 millions doses (strategic + pledged stocks). This is adequate to cover a range of scenarios. However, third generation vaccines are currently close to licensing and WHO hopes that once third generation vaccines become available, Member States will facilitate their incorporation into the stockpile.

Digitization of the Smallpox Eradication Programme Archives: Outcomes, perspectives and strategy

Marie Villemain Partow

World Health Organization

Background

The digitization project for the Smallpox Eradication Programme Archives started in June 2009. To date, the outcome has been very positive and presents many future possibilities.

Smallpox Eradication Programme Archives:

Physical Fonds:

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

Digitized Fonds:

Digitization:	Colour, 1/1, 300 dpi, OCR
Format :	TIFF and PDF/A
Size :	10 TB
Storage:	SATA2 discs on MD3000i, security copies on magnetic tracks LTO3 (EMC Networker)

Description and PDF/A files for consultation integrated into ERMS Livelink Enterprise Server.

Project Phases

Needs analysis	May–September 2009
Digitization	September 2009–August 2010
Integration into the database	5 months
Feasibility study	August 2009
Prototype	1 month (January–February 2010)
Cataloguing rules	1 month (March–June 2010)
Metadata entry	4 months (July–October 2010)

The outcomes

The main challenge of this project has been the volume of paper and electronic data involved. The team has benefited from the expertise of both internal and external specialists. This was the first large-scale digitization project undertaken by WHO Archives. Consideration was given therefore to longer term needs for future planning purposes in order to retain and re-use the knowledge and experience gained for future digitization projects. This project has demonstrated the importance of good planning and detailed needs analysis as well as the essential role of partnerships with external experts such as scientists in technical units and with IT.

The main scope was:

- preservation of the paper files
- integration of the scanned archives into a dedicated database with a powerful search engine

Both objectives have been achieved.

The strategy

Following the completion of the integration and preservation phase, the next focus is now to make the digitized files available to the public. WHO staff members will be able to access these files via a dedicated SharePoint site, in early 2011. All files within the smallpox archives have been digitized in pdf format, with a unique entry in a dedicated database. As a result, any file can be retrieved upon request for external and internal researchers. It is intended that all data will eventually be available worldwide via a dedicated web interface.

Annex 2. Agenda of the meeting

12th Meeting of the WHO Advisory Committee on Variola Virus Research, from 17 to 18 November 2010 Salle A, WHO, Geneva, Switzerland

Agenda

17th November 2010

9:00 - 9:15	Opening – Assistant Director-General for Health Security and Environment Election of chair & rapporteur
9:15 – 9:25	Report of the secretariat - WHO secretariat
9:25 – 9:35	Update on research proposals submitted to WHO and approved by the scientific subcommittee – R. Drillien
9:35 – 9:50	Update on Variola virus clones held at NICD, South Africa – B. Swanepoel
9:50 – 10:00	Summary of 2011 review process - WHO secretariat
	Presentation of the 6 chapters of the " Scientific Review of Variola Virus Research, 1999-2010 " (begin) 10mn +5mn discussion for each
10:00 – 10:15	Smallpox vaccines - Antonio Alcamí and Bernard Moss
10:15 – 10:30	Laboratory diagnostics - Inger Damon, Hermann Meyer, and Sergei Shchelkunov
10:30 – 10:45	Variola genomics - Grant McFadden, David Evans, Sergei Shchelkunov and Inger Damon
10:45 – 11:00	Tea/Coffee Break
	Presentation of the 6 chapters of the " Scientific Review of Variola Virus Research, 1999-2010 " (continued)
11:00 – 11:15	The status of WHO-CC repositories - Evgeniy Stavskiy, Christine Hughes, Inger K Damon
11:15 – 11:30	Animal models and pathogenesis - Peter B. Jahrling.
11:30 – 11:45	Antiviral drug development for Smallpox treatment - John W. Huggins and Nina Tikunova
11:45 – 12:15	Discussions on the 6 ACVVR review papers
12:15 – 13:15	Lunch
13:30 – 14:00	Presentation of the report of the " Advisory Group of independent experts to review the Smallpox research programme " - Tania Sorrell and Rakesh Aggarwal

14:00 – 14:30 Discussions of the report of the Advisory Group of independent experts to review the Smallpox research programme

14:30 – 15:00 Summary of discussions – R. Drillien/G.L. Smith

15:00 – 15:30 Tea/Coffee Break

15:30 – 16:00 The Subgroup Smallpox Laboratory diagnostic Network – J.-C. Piffaretti

16:00 – 16:15 The use of live Variola virus to maintain and regenerate non-infectious variola derived materials for diagnostic development support – K. Karem and V. Olson

16:15 – 16:30 2010 report on the variola collection of the WHO Collaborating Center Repository in VECTOR, Russian Federation – S. Shchelkunov

16:30 – 16:45 Discussion

16:45 – 17:00 The use of live Variola virus to evaluate antivirals against Variola – V. Olson

17:00 – 17:15 Animal Models and Pathogenesis: public health outlook – P. Jahrling

17:15 – 17:30 Update on ST-246 development – D. Hruby

17:30 – 17:45 Status of CMX001 development for Smallpox and other dsDNA Viruses - Randall Lanier

17:45 – 18:00 Clinical development status of the third generation non-replicating smallpox vaccine IMVAMUNE® – Lars Staahl Wegner

18:00 – 18:15 Update on the use of smallpox vaccine LC16m8 in Japan – H. Yokote and I Kurane

18:15 – 18:30 The use of live Variola virus to support less reactogenic vaccine development: continued evaluation of “third” generation vaccines – I. Damon

18:30 – 19:30 Social event

DAY 1 CLOSES

**12th Meeting of the WHO Advisory Committee on Variola
Virus Research, from 17 to 18 November 2010
Salle A, WHO, Geneva, Switzerland**

18th November 2010

9:00 – 9:15	WHO Smallpox Vaccines stocks, status update – WHO secretariat
9:15 – 9:45	Digitization of The Smallpox Eradication Programme Archives: Outcomes, perspectives and strategy – M. Villemin Partow
9:45 – 10:15	General discussion and preparation of draft meeting report
10:15 – 10:45	Tea/Coffee Break
10:45 – 11:15	General discussion and preparation of draft meeting report (continued)
11:15 – 12:00	Discussion about the future of the ACVVR
12:00 – 13:30	Lunch
13:30 – 15:00	General discussion and preparation of draft meeting report (continued)
15:00 – 15:30	Tea/Coffee Break
15:30 – 16:30	Final discussion and finalization of draft meeting report

ACVVR MEETING CLOSES

Annex 3. List of participants

12th Meeting of the WHO Advisory Committee on Variola Virus Research from 17 to 18 November 2010, Salle A, WHO Headquarters, Geneva

LIST OF PARTICIPANTS

TEMPORARY ADVISERS

MEMBERS OF THE ADVISORY COMMITTEE

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Representative for AMRO*

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Dr Faith McLellan, HSE/IHR, (Rapporteur)

* Unable to attend