WORLD HEALTH ORGANIZATION

EXECUTIVE BOARD
109th Session
Provisional agenda item 3.14

EB109/17
20 December 2001

Smallpox eradication: destruction of variola virus stocks

Report by the Secretariat

1. In May 1999, the Fifty-second World Health Assembly by resolution WHA52.10 authorized temporary retention up to not later than 2002 of the existing stocks of variola virus at the two current locations,[1] for the purpose of further international research. It also requested the Director-General:

> *(1) to appoint a new group of experts which will establish what research, if any, must be carried out in order to reach global consensus on the timing for the destruction of existing variola virus stocks, and will:*
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> > *(a) advise WHO on all actions to be taken with respect to variola;*
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> > *(b) develop a research plan for priority work on the variola virus;*
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> > *(c) devise a mechanism for reporting of research results to the world health community;*
> >
> > *(d) outline an inspection schedule to confirm the strict containment of existing stocks and to assure a safe and secure research environment for work on the variola virus, and make recommendations on these points;*
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> *(2) to facilitate the full participation in the work of the new group of experts of a limited number of scientists and public health experts from Member States of each of the WHO regions;*
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> *(3) to report the initial recommendations and plans of the group of experts, including relevant costs for WHO, to the Executive Board at its 106th session in May 2000, providing that external funding has been made available for this purpose;*
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> *(4) to present a detailed report, including progress of the research programme on the smallpox virus, to the Executive Board and Health Assembly as soon as possible, but in any event not later than 2002, and to make recommendations to the Executive Board and Health Assembly regarding their proposals for the date of final destruction of the remaining stocks of variola virus.*

[1] Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, and the Russian State Centre for Research on Virology and Biotechnology, Koltsovo, Novosibirsk Region, Russian Federation.

2. The WHO Advisory Committee on Variola Virus Research, composed of 16 members from all WHO regions and advised by some 10 scientific academic experts from such areas as public health, fundamental applied research and regulatory agencies, was subsequently established and has met three times. Reports from the first two meetings have already been submitted to the Health Assembly.[1] This document provides a report of the third meeting (Geneva, 3 and 4 December 2001).

THIRD MEETING OF THE WHO ADVISORY COMMITTEE ON VARIOLA VIRUS RESEARCH

3. The Committee agreed that, despite the considerable progress that had been made in investigating variola virus, significant components of this research, most notably the refinement and use of an animal model developed in 2001 and the development of antiviral drugs, were unlikely to be completed by the end of 2002. Further, during extensive discussion about the potential availability of an animal model, additional research was identified that would necessitate access to live variola virus stocks after the expected 2002 destruction date.

4. The Committee's main recommendation, therefore, was that serious consideration should be given to further extending the deadline for the destruction of variola virus in order to allow essential research to be completed. Further, this additional research with live variola virus should continue to be carefully monitored and reviewed under the auspices of WHO, and steps should be taken to ensure that all approved research would remain outcome-focused and time-limited and periodically reviewed.

5. **Review of variola virus strains in the two repositories.** It was previously noted that the Centers for Disease Control and Prevention held 451 viral isolates obtained from different continents and countries when smallpox was endemic.[2] The current review and the studies reported at the meeting concentrated on some 50 isolates in the Russian collection that were not present in the American collection. From these isolates, 23 strains from scab material and previously lyophilized samples were viable in tissue-culture. Isolation of DNA from these strains is continuing; already, two genomes have been completely cloned and at least five others will be cloned by the end of 2002. The Committee agreed that before the end of 2002 further consideration should be given to the necessity of holding the wide range of isolates currently available in the two repositories.

6. **Nucleic acid-based diagnostics.** Several methods have been devised recently for very sensitive detection of variola virus DNA and to distinguish this DNA from that of other orthopoxviruses, the most promising being analysis by polymerase chain reaction (PCR) of restriction fragment length polymorphisms, multiplex PCR and real-time PCR with fluorigenic probes. Some of these tests have been used in the definitive diagnosis of a recent laboratory-acquired infection with a non-variola orthopoxvirus.

7. The results obtained indicate that single-gene restriction fragment length polymorphisms and multiplex PCR detection methods are useful for detecting variola virus in clinical samples. The Committee noted that, although the real-time PCR test has greater sensitivity and can therefore detect infection at an earlier stage, it requires the use of expensive equipment and, so far, cannot consistently distinguish between species of orthopoxviruses. An extended PCR test for restriction fragment length

[1] Documents A53/27 and A54/16.

[2] Document A54/16.

polymorphisms has proved useful in defining the origin of an isolate, but may require prior tissue-culture passage of clinical samples.

8. The Committee recognized the significant progress made in the area of molecular diagnosis but agreed that there was still scope for improving the sensitivity of the tests available. For example, it would be useful to know how early infection with variola virus might be detected at the prodromal stage. An ultimate goal might be the development of relatively cheap hand-held equipment for the detection of variola virus DNA and diagnosis of infection.

9. To further this important area of work, the Committee encouraged investigators to share diagnostic reagents, essential primer sequences for PCR assays and protocols where appropriate. This cooperation would be particularly useful for enhancing capabilities in different countries for the rapid and reliable detection and diagnosis of variola virus infections.

10. **Sequence analysis of variola virus DNA.** The Committee was informed that the complete genomes of an additional seven isolates of variola virus had been sequenced, bringing the total number of full-length genome sequences to 10 (nine variola major and one variola minor strain). The sequences were highly conserved. To counter the criticism that this result was a consequence of tissue-culture passage, the Committee suggested that further thought be given to sequencing DNA directly from scab material. The known degree of virulence of isolates has not yet been correlated with identified variations in sequences.

11. The Committee noted that a considerable amount of information on the nucleic acid sequences of variola viruses was now available. After discussion, it was agreed that further sequencing of the more variable genomic termini had priority over the derivation of sequences of additional whole genomes. This would be useful for forensic purposes if there were ever a deliberate release of variola viruses, and reference DNA should be kept for this purpose.

12. **Serological assays.** Polyclonal and monoclonal antibodies against vaccinia virus have been used in various enzyme-linked immunosorbent assays to evaluate their usefulness in the detection of variola virus antigens. Polyclonal antibodies detected all viral strains more readily than the monoclonal antibodies currently available, but, although the methods appear to be relatively sensitive, they do not facilitate the detection of all viral isolates. The Committee concluded that a variola virus-specific serological assay could usefully complement molecular diagnostic techniques, particularly as a second method to detect infection. However, further validation of the tests available was needed.

13. **Animal models.** The Committee was informed of the successful infection of cynomolgus monkeys with two different variola virus strains by intravenous, or intravenous plus aerosol, routes. The disease induced shared several pathological features with human smallpox but the course of disease is much faster and the dose of virus needed to cause intravenous infection is particularly high.

14. Additional studies are needed to improve and validate this animal model, but this would need work extending beyond 2002. The monkey model has the potential to be used as an assay in prophylactic or therapeutic studies with live variola virus and could also provide access to good diagnostic reagents. Other surrogate animal models are being investigated in parallel, in particular the infection of monkeys with monkey pox virus and the infection of rodents with cow pox virus, in order to obtain data that relate more to models using variola virus.

15. **Drug development.** Most studies have focused so far on the efficacy of cidofovir against pox viruses. This compound has demonstrable activity against cowpox in mice and against monkeypox in

monkeys. In the United States of America cidofovir may be used in emergencies as an investigational new drug to treat significant adverse events following immunization with the current smallpox vaccine, and in the unlikely event of smallpox re-emerging.

16. *In vitro* screening of other chemical entities has identified more than 140 additional compounds with antiviral activity against pox viruses. The finding that some of these compounds have selective activity, inhibiting one or more orthopoxvirus but not necessarily variola virus, supports the premise that access to live variola virus is necessary for the effective screening of additional lead compounds. Most active compounds identified so far target the viral DNA polymerase and it was considered important to identify other viral gene products susceptible to drug intervention.

17. **Vaccine development.** The Committee agreed that the best safeguard against smallpox was vaccination. This strategy had been successfully deployed during the eradication programme, but the smallpox vaccine currently available was associated with a significant number of adverse events. This suggested that, although the current vaccine had proved its efficacy and utility, improvements were needed, particularly to facilitate the safe and effective immunization of vulnerable sectors of certain populations (the immunocompromised, the elderly, pregnant women and children with eczema).

18. The Committee therefore encouraged the delineation of further research into vaccine strategies that might use more attenuated vaccinia virus strains, subunit vaccines or other promising approaches, including DNA vaccines. Results reported at the meeting and in numerous publications on attenuated vaccinia virus recombinants encoding antigens from other pathogens indicate the potential value of these alternative strategies for vaccine development. It was recognized that access to live variola virus would be necessary to assess the efficacy of new, improved smallpox vaccines and, ultimately, to obtain regulatory approval.

19. **Conclusions and recommendations.** The Committee acknowledged that important progress has been made in health-oriented research involving variola virus. However, it concluded that much essential research will not be completed by the end of 2002. The Committee recommended that further goal-oriented research, extending beyond the expected 2002 destruction deadline, could be justified so that the world population could be adequately prepared for the unlikely, but potentially catastrophic, event of a re-emergence of smallpox.

20. It was further recommended that the current advisory committee should continue its role in monitoring and reviewing all research involving live variola virus, and that steps should be taken to ensure that all approved research would remain outcome-focused and time-limited.

RECOMMENDATIONS OF THE DIRECTOR-GENERAL

21. Having noted the report of the Advisory Committee for Variola Virus Research, including the recommendations for research priorities, and its conclusion that the research programme will not be completed by the end of 2002, the Director-General recommends that:

- the WHO Advisory Committee on Variola Virus Research should continue to oversee the variola virus research programme and that the research programme should be conducted in an open and transparent manner;

- the research programme should be completed as quickly as possible, and a proposed new date for destruction should be set when the research accomplishments and outcomes allows consensus to be reached on the timing of destruction of variola virus stocks;

- regular biosafety inspections of the storage and research facilities should be continued in order to confirm the strict containment of existing stocks and to ensure a safe research environment for work with variola virus;

- depending on progress, a report on the research should be submitted to the Executive Board and Health Assembly in two to three years' time.

ACTION BY THE EXECUTIVE BOARD

22. The Executive Board is invited to note the report and to endorse the recommendations of the Director-General.

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