INTERNATIONAL WORKSHOP ON DEVELOPMENT OF VACCINES FOR SARS AND NEW HUMAN INFLUENZA VACCINES

SUMMARY AND CONCLUSIONS

CO-SPONSORED BY

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AND

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Executive Summary

The Ministry of Science and Technology of China and the World Health Organization held a joint workshop on the development of vaccines against SARS and avian influenza. The workshop was attended by over 60 experts – scientists, physicians and ethicists - from China and around the world. The week included parallel meetings and visits to several institutions, related to various aspects of the vaccine programmes on SARS, Influenza, HIV/AIDS, Japanese Encephalitis and Malaria. All this has allowed for a fruitful exchange of information and views on vaccine development in China.

Progress reported on SARS vaccine development

China has achieved much progress towards the development of the first-ever vaccine against SARS. Pre-clinical, laboratory-based studies involving animals have been carried out, and an application has been made to the State Food and Drug Administration (SFDA) for authorization to begin a Phase I clinical trial of this vaccine. A Phase I trial may begin as early as April or May 2004.

Participants in the workshop reviewed some of the data from the pre-clinical trials involving animals, and further steps were discussed towards refining methodology and data requirements to launch a Phase I trial in humans.

China has invested considerable effort and resources in the quest to develop a SARS vaccine, marshalling the large scientific research potential of the country. It normally takes several years from pre-clinical testing through pharmaceutical and clinical development to availability of a vaccine for the general human population. China has mobilized its scientific forces with great energy to put SARS vaccine on the fast track. Still, it is unlikely that a vaccine will be available before two to three years, underscoring the compelling need for sustained surveillance, early diagnosis of suspected cases, effective case management and outbreak containment activities.

Developing an avian influenza vaccine for humans

Even as measures continue to be applied to help curb the ongoing outbreaks of **highly pathogenic avian influenza** in poultry in the Asia-Pacific region, international efforts are well underway to develop an avian influenza vaccine for humans.

In China, as in other countries affected by these outbreaks, efforts have focused on the isolation and characterization of viruses – an essential first step before embarking on vaccine development. China is sharing live virus isolates with WHO international reference laboratories as part of this international effort.

WHO has reported in recent days that the development of an avian influenza virus prototype for a possible vaccine appears to be on schedule, after being tested in animals. It is possible that the "prototype virus" could be available by the end of this month. It would then be offered to various vaccine manufacturers around the world, including in China, who could produce clinical batches for Phase I testing in human volunteers.

Vaccine development for Malaria and HIV/AIDS

A vaccine for **malaria** would also be an enormously useful public health tool. The disease is one of the three major killers of children, causing more than 1.1 million deaths each year. Yet, a vaccine for malaria has never been successful developed, nor has the disease been properly controlled. Some of the workshop delegates visited the Shanghai Hospital and the 2nd Military Medical University to discuss ongoing research into a malaria vaccine.

There are several ongoing projects on **HIV/AIDS** vaccines in China, some in collaboration with European or US-based institutions. The diversity of these projects calls for greater harmonization in HIV/AIDS research. To this end, China has produced its first National Plan on HIV/AIDS Research. This topic has increasingly captured the attention of China's leadership and stimulated commitment towards financial and scientific support in developing a vaccine.

Ensuring vaccine quality, procurement and supply is a continuous process. The strengthening of the National Regulatory Authority is essential to ensure the quality of locally produced and/or imported vaccines. This will help ensure their quality, potency and safety. China State Food and Drug Administration and WHO look forward to working together in assuring vaccine quality of the highest international standards.

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I. Introduction

At the invitation of the Chinese Ministry of Science and Technology and the World Health Organization, scientists from nine countries met to discuss the state of the art of development of vaccines for SARS and new human influenza vaccines.

The experts were welcomed by Guoming Qi, Director General of the Ministry of Health, who spoke of the profound and negative impact that SARS has had on many countries and regions, and who emphasized the crucial importance of the research and development of a SARS vaccine. Dr Guoming pointed out that SARS has become one globally key scientific and technological project.

The workshop was then addressed by representatives of the organizing agencies.

Professor Yu Wang, the deputy Director General of the Ministry of Science and Technology, presented a scientific overview of research and development of SARS and Avian influenza. Professor Wang expressed the hope that China would be considered as one of the major clinical trial bases for HIV vaccine, and that the workshop would initiative a global protocol for clinical trials of SARS vaccines.

The WHO Representative in China, Dr Henk Bekedam, congratulated the commitment and collaboration of the Chinese Government towards tackling disease; such commitment is paramount in combating emerging infectious diseases. Dr Bekedam identified the focus of the workshops and visits as being SARS, Avian influenza, Malaria and HIV/AIDS, and emphasized the essential role of the National Regulatory Authority in assuring vaccine quality of international standards.

The workshop included more than 60 experts in virology, immunology, clinical trials, bioethics, as well as regulators and producers of vaccines. The broad range of participants illustrated the importance of international collaboration in dealing with emerging public health threats. It was stressed that vaccine development approaches, adhering to internationally recognized standards, will benefit not only public health but also facilitate access to international markets for a successful product. The list of participants appears as Annex 1.

II. Monday 1st March. Plenary Session 1: Regulatory aspects of vaccine research and development

Assurance of the quality, safety and efficacy of a vaccine requires specialist regulatory oversight. Approaches to the regulation of candidate SARS vaccines in the USA, China and Europe were described. There were considerable similarities between approaches. All regulatory authorities, for example require that the products be characterized to demonstrate suitable quality, purity, potency and safety; that clinical trials be done under appropriate regulatory supervision; that they be ethically conducted; and that adverse events be monitored in a suitable way. The guidelines used by the different regulatory agencies were listed and, in general, were stated to comply with WHO guidelines. In addition, China has produced the first guideline on production and quality control specifications for inactivated SARS vaccine candidates. A special concern in the non-clinical evaluation of inactivated SARS vaccines is to test for the possibility of immune enhancement of disease, which has been observed with experimental veterinary vaccines against the feline infectious peritonitis coronavirus.

Each of the regulatory authorities described fast track mechanisms that could be evoked in the face of a public health emergency to evaluate potential interventions. For SARS vaccines, the fast-track procedure had been initiated in China in 2003 as a response to the urgent need to be prepared, in case a new SARS epidemic occurred in the early months of 2004. To date, no new epidemic had been detected and so it seemed legitimate to proceed with caution, especially as there is more to be understood about the basic biology of the SARS CoV.

New diagnostic tests for SARS CoV were described. Since the clinical case definition of SARS is not specific, laboratory diagnostic tests are needed for clinical management decisions, public health and epidemiological purposes. In the context of vaccine trials, diagnostic tests are needed to pre-screen clinical trial volunteers and to

monitor specific responses to the candidate vaccine. Given that the spectrum of coronaviruses infecting humans may not yet be fully understood, the specificity of diagnostic tests needs especially careful consideration. Further work is also needed on the sensitivity of tests, especially to determine if viruses which differ from prototypic SARS coronavirus strains could be detected. The sensitivity of virus detection methods may be affected by the type of sample examined; since the SARS CoV replicates mainly in the deep respiratory tract, samples from the upper respiratory tract may be sub-optimal. At the present time, the gold standard method for virus detection is considered RT-PCR, although confirmatory testing is always required, preferably in a WHO International SARS Diagnostic Reference laboratory. For serological assays and monitoring sero-responses in vaccine trials, the neutralizing antibody test is the gold standard, although other methods such as ELISA, may be used for screening purposes.

Conclusions of this session

1. Emerging threats to the public health, such as SARS or H5N1 influenza viruses, require coordinated international actions for control and prevention. To be effective this requires openness, sharing of data and materials, and international scientific collaborations.

2. In the area of vaccine development against SARS, very rapid progress has been made in preparing candidates for clinical trials. This rapid progress presents challenges to regulatory authorities worldwide to assure the quality, safety and efficacy of the candidate vaccines. International collaboration between regulatory authorities is encouraged to develop common standards for quality, safety and efficacy.

3. Regulatory authorities have fast-tracked mechanisms for products required in public health emergencies. The justification for fast-track assessment may change as the epidemiological situation changes. For SARS vaccine, given the apparent lack of a new outbreak in 2004, it is appropriate to proceed with caution for the continued assessment of candidate SARS vaccines.

4. Diagnostic methods for SARS need further development, particularly to establish specificity to discriminate from other human coronaviruses. The gold-standard method for detection of SARS CoV is, at present, RT-PCR and for detection of antibodies to SARS CoV is the neutralization test.

III. Monday 1 March. Plenary Session 2: Product development issues

The session reviewed progress in the development of SARS vaccines and discussed issues relating to manufacturing, scale up of production, quality control and pre-clinical evaluation of candidate SARS vaccines.

A major focus of presentations was on production and pre-clinical evaluation of whole inactivated SARS vaccines. The Chinese SARS vaccine programme is one of the most advanced in this field. The development of a whole inactivated SARS vaccine by Sinovac Biotech Co. was presented. This candidate vaccine is based on the SARS CoV strain Sino 3 (a Chinese isolate obtained from a SARS patient in Beijing in 2003), grown in Vero cells, inactivated with beta-propiolactone, purified and adsorbed on Alum. The vaccine strain has been characterized, including full-length genome sequencing. Production facilities were established to meet required national and international BLS3 standards as well as GMP norms for the production of biological material for human clinical trials. For the purposes of quality control, the research group has established and validated in-house reference materials and assays, including SARS reference antiserum, calibrated SARS virus antigen, titrated SARS virus (Sino1) stocks, as well as neutralizing and potency tests. Safety and immunogenicity of this candidate vaccine were evaluated in different animal species, including Balb/C mice, guinea pigs, rabbits and rhesus macaques. Following challenge the immunized macaques with virulent SARS CoV (Sino 1 strain), no protection against viremia was observed (by RT-PCR), but protection against interstitial pneumonia seemed to correlate with the presence of neutralizing antibodies before challenge. Two lots of the candidate vaccine have been controlled by the Chinese National Institute for Control of Pharmaceuticals and Biologicals and approved by the State Food and Drug Administration (SFDA) for testing in human clinical trials.

A second Chinese candidate vaccine against SARS is being developed by research groups of four institutions (Epidemiology and Virology Institute, National Vaccine and Serum Institute, Beijing Genomics Institute and Harbin Institute of Veterinary Medicine). The vaccine is based on the SARS CoV strain BJ01, grown on Vero

cells, inactivated with beta-propiolactone and purified by ultra-centrifugation, gel-filtration and ion-exchange chromatography. Three batches of the candidate vaccine have been tested for quality, safety and immunogenicity. Vaccine potency was determined in mice, guinea pigs and rabbits. Vaccine efficacy was further evaluated in challenge experiments in macaque monkeys. Preliminary results indicated that vaccinated monkeys seemed to be protected against severe interstitial pneumonia (as assessed by histopathological methods), but not against viral infection.

Technologies for large-scale production of whole inactivated SARS vaccine are being developed by two industrialized country pharmaceutical companies (Baxter Vaccines and Aventis Pasteur, under contracts from the USA NIH) which have experience in production of other viral vaccines. One approach presented is based on manufacturing an inactivated SARS vaccine (Utah strain), using Vero Cells Microcarrier Cultures in large 1200L biofermenters. The production facility was already approved for production of other viral vaccines. Based on this technology two vaccine approaches are being pursued, including whole-inactivated and vectored vaccines (MVA-S glycoprotein, VV-S glycoprotein). Each production stage has been fully characterized and controlled. For the SARS inactivated vaccine, the company has optimized its production by vaccine strain clone selection, optimization of growth kinetics, inactivation process, purification, development of control methods to evaluate vaccine safety and immunogenicity. In-depth research and development is being conducted by another company also aimed at large-scale production of an inactivated SARS vaccine. The key issues in the production process were described as (1) establishment of BSL3(+) facilities required for working with the SARS CoV; (2) detailed characterization of the vaccine (Utah strain), (3) inactivation procedures, (4) virus stability after purification and inactivation; (5) batch release testing; (6) immunogenicity studies, including choice of appropriate animal models for dose ranging studies.

Another vaccine production approach was presented, which is based on the use of a *Baculovirus* Expression Vector System (BEVS) to produce a sub-unit S-glycoprotein SARS vaccine (Protein Sciences). A number of advantages of this technology were described, including versatility, speed and safety profile. However, limitations of this system were also identified, such as absence of vaccine products on the market produced by this technology, simple glycosylation patterns, as well as some intellectual property rights limitations. The development of a SARS vaccine through this approach focuses primarily on the SARS S-glycoprotein because this protein is expected to induce antibodies with virus neutralizing properties. Preliminary studies in mice demonstrated the ability of the antigen to induce virus-neutralizing antibodies. Clinical trials of this vaccine candidate are envisaged as early as 2005.

The development of SARS immunotherapy approaches using human monoclonal antibodies (HuMab) was presented. This approach, supported by a grant from NIH, could serve as a proof of concept for the development of SARS vaccines targeted at induction of humoral immunity. In addition, availability of effective immunotherapeutic agents would allow for the development of prevention strategies for post-exposure prophylaxis. The research group is attempting to identify viral epitopes responsible for induction of protective neutralizing antibodies. Multiple virus neutralization epitopes were identified on the S-protein, in particular one major epitope was mapped in the region located between 270-510 amino acid sequences in the S-protein. Transgenic mice producing humanized antibodies were immunized and screened for S-specific humoral responses. The researchers are in the process of establishing a library of hybridomas producing anti-SARS antibodies with neutralizing activity. Two scenarios for testing these products in animal studies and human clinical trials were discussed, suggesting different approaches in the two following situations: a) new cases of SARS are detected (fast track) and b) the SARS epidemic does not re-emerge in the near future (slow track). It was noted that these trials will need to address a number of important issues, in particular those related to a choice and use of placebo, if deemed ethical.

Conclusions of this session

1. Substantial progress has been made in the area of SARS vaccine development. The front-line research efforts are primarily focused on the development of whole inactivated candidate vaccines. However, parallel development of other vaccine strategies based on the latest vaccine strategies and technologies and targeting cellular arm of immune responses should also be encouraged.

2. Several whole inactivated candidate vaccines are under development and have reached advanced stages of pre-clinical testing. The Chinese SARS vaccine programme is the most advanced in this area, with at least 2 different vaccine candidates being readied for human testing.

3. The key challenges for large-scale production of whole inactivated candidate vaccines will be to ensure high level standards of GMP, Biosafety (BSL3), quality, safety and potency control procedures. The development of national and international/WHO guidelines for these purposes will be important.

4. Laboratory methods for testing of quality, potency and safety of SARS candidate vaccines will need to be further developed and standardized. For these purposes, there is an urgent need to develop and make freely available common protocols, national and international reference materials, including panels of well characterized HuCoV strains, polyclonal and monoclonal binding and neutralizing antibodies, reference viral antigens etc.

5. Animal models for testing of SARS vaccine studies on safety, immunogenicity and potential efficacy should be further developed and standardized, including the development of standardized and titrated stocks of SARS CoV strains for challenge studies on homologous and heterologous protection in different animal species.

6. A major safety concern with SARS vaccines is related to potential immune enhancement of infection or disease in vaccinees. Further efforts should be made to develop *in vitro* and *in vivo* assays to evaluate potential vaccine-related immune enhancement.

7. The decisions of the national regulatory authorities with regard to approval of phase I human clinical trials with new candidate SARS vaccines should be based on careful review and assessment of all available data on every stages of vaccine production, products quality and safety control, as well as data from animal studies.

IV. Tuesday 2 March. Plenary Session 3: Clinical Trials

This session touched upon a number of general issues related to the design and conduct of vaccine clinical trials, as well as upon the design of future phase I trials of a SARS vaccine candidate.

There are a number of ethical issues to consider when designing a vaccine trial. Clinical trials have to adhere to rigorous ethical guidelines and should be statistically sound, taking into account biases (internal and recruitment), confounding factors, the length of trials and thus drop-outs as well as statistical power. The standard of follow-up care for trial participants has to be agreed upon before the trial takes place (the best care available in the country or the highest attainable care in the world; this issue has not yet been resolved). A process should be developed to involve and inform community about the research before, during and after the trial, and a local ethical review committee must be established.

Vaccine efficacy as well as effectiveness should be measured in phase III trials. Post-marketing or adverse event surveillance is also vital (through active or passive surveillance or through *ad-hoc* studies). A surveillance system has been prepared by China to ensure monitoring of adverse events. Pilot studies in neighbouring countries are underway and these models may apply well to China.

Meeting participants were reminded of the main ethical considerations in vaccine clinical trials. These specify that healthy human subjects may be harmed so protection and safeguards are needed. Therefore, treating subjects without thought for their health and well-being and merely as a means for research is ethically wrong. It is of importance to note that research can be beneficial and may provide advantages in health care to participants who

would otherwise not have access. The issue of Standard of Care for trial participants who become infected through the trial was raised. Finally, human subjects should not be exploited and there should be a realistic plan to make effective vaccine products available to the study population and/or country where the trial has taken place.

Application of ethical principles has a number of consequences. In particular, participants must be recruited in a respectful manner, and should be well informed about the nature of the research and potential future benefit to others. They must also be informed about the risk of harm. In addition, volunteers should be aware of confidentiality issues. It is important to remember that ethical principles are universal, but the mechanisms and procedures applying principles may vary.

A presentation was further given on statistical concepts applied to the design of clinical trials, which included such issues as number of subject who should participate in the study, consequences of participants withdrawal, confounding factors and control for biases.

The rationale and design for randomized double-blind clinical trials (RCT) and case control studies to analyze the effectiveness of vaccines were compared. Case control studies represent a less expensive way to conduct a study to assess effectiveness if a RCT design is not possible. Case controls studies attempt to control for differences between control and intervention groups that may confound estimation of the vaccine effect. There are several ways to ensure or account for bias, in the enrolment phase, when actually conducting the study and when analysing the results. However, to measure the actual practical confounding factors is difficult. Specific examples were discussed.

Participants were made aware that post marketing surveillance/assessment of adverse effects following immunization is essential. RCT might be under-powered to detect rare effects, and continued monitoring of adverse effects is therefore crucial. In addition, pre-licensure trials may not be able to detect rare adverse effects as they enrol a narrow spectrum of subjects and are performed outside the complex concept of concurrently administered vaccines and other medications that occur in the real life settings. The pre-licensure trials also fail to detect events that occur long after vaccination. Monitoring of adverse event following immunization (AEFI) is therefore necessary. *Ad-hoc* studies to detect adverse events are often used but have a number of limitations, mainly availability of data, costs and time. Systematic surveillance, be it passive or active, can avoid some of the limitations of *ad-hoc* studies. So, signals of the presence of AEFI can be detected through passive reporting systems and are inexpensive. However, they are difficult to standardize, can be biased, with no clear denominators and a lack of data in the comparison group. It is necessary to create systems to record adverse events in developing countries including China. It was reported that the creation of large database to record adverse events is being conducted as a pilot study in Vietnam. This model might be useful in China.

Conclusions of this session

1. A process should be undertaken to establish guidelines for the standard of care for trial participants who become ill during the course of a trial.

2. WHO needs to produce generic vaccine clinical trial protocols to act as guidelines for minimum level of contents in protocols.

V. Parallel Session 1: Roundtable on Clinical trials

During the roundtable, issues related to protocol and trial design, fast-track licensure, Good Clinical Practice (GCP) and Standard of Care were discussed.

Before commencing a vaccine trial a complete protocol has to be submitted. Trials that adhere to the principles of GCP should be scientifically sound, ethical, and conducted in line with the clinical protocol. Quality control procedures should be implemented for every aspect of the trial (SOPs, training and verification of training) and data collected should be handled properly. Quality assurance should also be conducted through compliance monitoring (spot checks) or monitoring by regulatory authorities. The products should be handled, manufactured and stored in accordance with GMP and they should be used in accordance to the protocol.

When recruiting and informing trial participants for any trial, an informed consent form is a requirement. Language used should be comprehensible to a lay person and void of medical terminology. The form is also used to clarify rights to compensation in case of adverse events, as well as anticipated risks and benefits.

One issue which was brought up was whether samples taken during clinical trials can be used for other research. The consensus is that if the samples can be traced back to the individual either directly or indirectly, then it is necessary to ask that individual for consent. If this is not possible, if the study cannot be conducted without samples and if this represents a significant study, then the samples may be used. Failure to give informed consent should not place the subject at risk.

Clinical trials should be conducted within the principles of the Declaration of Helsinki, and are submitted to approval by an ethical review committee (ERC). The role of the ERC is to ensure that ethical principles are applied, to determine if the risks are reasonable and that the benefits outweighs the risks. They ensure equity in the search for research participants, as well as non-coercive enrolment and compliance with national laws. They also monitor research in progress.

Documents to be submitted to an Ethical Review Committees include:

- A complete scientific protocol
- Investigators brochure (if any)
- Progress reports (once the study is underway)
- Adverse events (both in single site and multisite studies - include events from other sites)
- Literature review with references
- Data from preclinical studies, earlier phases of this study
- The Informed Consent form
- The hypothesis of the study
- Objectives
- Methods section
- Statistical analysis
- Inclusion/exclusion criteria
- Criteria for withdrawing subjects from the study and stopping the study as a whole
- Details about the recruitment of the study - who will recruit, where and when.
- Anticipated risks (risks of harm), including discomfort (e.g. pain)
- Expected benefits (direct and indirect)
- Risk/benefit ratio
- Protection of confidentiality (who has the data, how long will it be kept…)
- Whether human biological samples will be taken, will they be kept, used or destroyed
- Who will obtain the informed consent
- Reasons for discontinuation of the trial should be stated

Below is a consensus list of items which were described as the minimum information to be included in a informed consent form:

- Purpose of study;
- Procedures to be performed;
- Identification of which procedures are experimental and which are routine clinical care;
- Known or anticipated risks of harm, including psychological, social, legal, discomfort, inconveniences;
- Monetary compensation in case of injury - by insurance or other;
- Anticipated benefits (to the subjects and others);
- Whether biological samples will be taken and, if so, how long will they be stored;
- Information on whether genetic studies will be done on samples;
- Protection means to ensure confidentiality;

- Alternatives to subjects participation;
- A statement that participation is voluntary, that subjects may withdraw at any time without prejudice for their future care, treatment or other interests;
- A statement that any new scientific information that may affect willingness to continue in the study will be shared with the subjects;
- Whether subjects will be paid, if so how much;
- Whether subjects will incur any costs.

During this session the issue of standard of care was presented and discussed. HIV vaccine clinical trials were as a starting point for discussion and the recommendations that were made during a WHO consultation on this topic in June 2003 were presented (see Report of the consultation).

Conclusions of this session

1. WHO should provide technical assistance to China towards improving the Phase I protocol for the clinical trial of the SARS vaccine candidate(s). This will ensure that clinical evaluation is performed following internationally recognized standards of Good Practices, but not imply endorsement of the protocol by WHO.

2. WHO will undertake the production of guidelines for writing protocols on clinical trials for products targeted at rare pathogen with high public health significance.

3. In consultation with scientists from China and other concerned countries, WHO will produce a generic protocol for the phase 1 clinical trial of SARS vaccine candidate(s).

VI. Parallel Session 2: H5N1 Influenza Vaccines

The purpose of the session was to present and discuss the diagnosis and epidemiology of H5N1 Influenza ("bird flu") and the current status of development of vaccines against the disease.

The terms of reference and achievements of the Task Force on Bird Flu in China were presented. The concern about avian influenza and its transmission to humans have engendered a willingness of the Chinese authorities to step up coordinated actions between different ministries into the fields of epidemiology and epizoology, diagnostics and vaccine production for avian influenza.

The work initiated by WHO in response to the recent outbreak of H5N1 influenza and the transmission of the virus to humans was presented. The WHO mechanisms of identifying strains with a pandemic potential, and subsequent strain selection for vaccine production were situated in the framework of the WHO pandemic preparedness plan. The issues relating to the use of the new technology of reverse genetics to facilitate the production of vaccine strains were discussed. Such issues include advantages as to viral safety and rapid vaccine strain availability and difficulties relating to the commercial use of this patented technology and to specific regulations of Genetically Modified Organisms.

The current development of vaccines against H5N1 in China includes all types of vaccines (inactivated whole and split virion as well vaccines prepared by recombinant technology). The importance of adequate vaccine strain selection was emphasized, using criteria including cross-immunity and protection, virulence and adaptation to culture systems. The technology of reverse genetics to produce reassortants is being used to produce vaccine strains. The question of how to evaluate vaccine efficacy in the absence of human cases in China was raised.

A flexible large-scale vaccine production process using a continuous mammalian cell line and serum-free growth medium was presented. GMP-licensed BL3 facilities were available allowing the use of wild type influenza isolates for vaccine production. The advantages of such a system included fast availability of large quantities of vaccine independent of the availability of large amounts of embryonated eggs. The system had been found suitable for a wide range of human and avian influenza strains. The experience with adjuvanted vaccines with reduced antigen content was also presented.

The importance of the availability of diagnostic reagents with high sensitivity and specificity was underlined. Diagnostic tools included RTPCR, IFA, HAI and micro-neutralization. Whereas Nucleic Acid Amplification methods were considered to be the most efficient diagnostic tools, the need for developing less expensive tools of equivalent performance was underlined.

The EU regulatory aspects of influenza pandemic preparedness as to vaccines production were presented. The EU procedure for making available a vaccine of adequate quality, safety and efficacy was outlined. It is based on the approval of a model ("mock-up") vaccine in the interpandemic period, followed by and a fast-track approval of the pandemic vaccine, when required.

The genetic evolution of influenza viruses was discussed. Genetic differences were observed between e.g. the Hong Kong 2003 isolates and the current H5N1 strains from human cases isolated e.g. in 2004 in Vietnam, which led to reduced serological cross-reactivity. Concern was expressed about the unprecedented and continued human exposure to both human and avian strains circulating in the Asian region. The need for more studies into the animal reservoirs and into the mechanism and frequency of transmission to humans was underlined.

Conclusions of this session

1. The continuing epidemic of avian influenza poses a threat for animals and humans alike. The threat is potentially of a global dimension. Considerable progress has already been made in the field of diagnosis, epidemiology, epizoology and vaccine development.

2. Further efforts are needed to improve the tools such as diagnostic reagents and vaccines.

3. Further studies into the animal reservoirs and the mechanisms of transmission of the virus to humans are needed.

VII. Parallel Session 3: SARS Animal Models

The focus of the session was on animal models of SARS CoV infection. Experience of the participants with various animal species was reviewed: *Macaca fascicularis, Macaca mulata,* cats, ferrets, civet cats, rats, hamsters, guinea-pigs and mice.

Importantly, several research groups had reproduced results published in 2003 on the sensitivity of non-human primates to SARS CoV infection. Indeed, both macroscopical and histological lesions were demonstrated reproducibly in the lungs of SARS CoV-infected monkeys (both cynomolgus and rhesus). Virus was detected in infected animals starting at day 2 (cynomolgus) or day 5 (rhesus). Therefore, the importance of using early time points after infection to analyze viremia and histopathological changes was underlined. Day 5 after challenge was suggested for cynomolgi (and ferrets) and day 7 for rhesus. In addition, mild pathology (elevated temperature) was observed in some monkeys. Infected animals developed both antibodies and CTL to SARS CoV antigens.

Experimental challenge of civet cats with two strains of SARS CoV was presented, demonstrating the high susceptibility of this animal species towards the SARS virus.

An experiment was reported which included guinea pigs, adult mice, suckling mice, hamsters, rats, chickens, as well as pigeons, experimentally infected with SARS CoV (BJ01 strain) by the intranasal route (intracranial route for suckling mice). No histopathological or clinical manifestation was observed in any of these animal species, but antibodies to SARS CoV proteins were detected. Virus was detected by RT-PCR in the lungs of only guinea pigs and rats. The same research group also experimentally infected *Macaca fascicularis* and *Macaca mulata* monkeys. No clinical signs were observed. Interstitial pneumonia was present and virus detected in various specimens by RT-PCR.

Two groups reported immunization and infectious challenge studies in non-human primates. In the first report, rhesus macaques were immunized with 32SU (10 ug) of Sino-3 BPL inactivated candidate vaccine at Day 0 and 14 or Day 0 and 28. In these immunized animals, no protection against viremia was observed (by RT-PCR), but protection against interstitial pneumonia (as detected by histochemistry) seemed to correlate with the level of neutralizing antibodies before challenge. The second group (from Wuhan) reported experiments in rhesus and cynomolgus monkeys. Animals were immunized with escalating doses (0.5, 5, 50 and 5000 ug protein) of NS-1 SARS CoV strain (Ningxia province isolate) BPL-inactivated SARS candidate vaccine at Day 0 and 7. This vaccine had been purified by gel filtration and exchange chromatography. Animals corresponding to the 5000 ug dose were sacrificed and examined for any sign of pathology. No clinical change was recorded in the monkeys. Animals corresponding to the 0.5, 5 and 50 ug doses were subsequently challenged and appeared to be partially (0.5 ug) or completely (5 and 50 ug vaccine) protected against SARS CoV infection (no virus detected by RT-PCR).

A report was also given on the February 2004 meeting on SARS animal models organized by WHO and Erasmus University in Rotterdam.

Finally, safe manufacture of SARS inactivated vaccines under BSL-3 containment was discussed, as well as the general biosafety requirements and specialized regulatory constraints for production under Good Manufacturing Practices (GMP). A parallel was drawn with containment measures which will be required for wild poliovirus-based vaccines in the post-eradication era.

Conclusions of this session

1. More work is needed to define and standardize the best animal models.

2. SARS vaccine efficacy testing requires demonstration of:
   - abrogation of disease or decreased pathology and histopathology
   - decreased viremia
   - appropriated immune response

3. Safety testing of SARS vaccines would require:
   - a test for disease enhancement in both early and late challenge models
   - if possible use of at least two animal models (rodent and non-human primate)

4. Both safety and efficacy studies should be undertaken ensuring standardization and adequate statistical power

5. WHO recommends national governments to maintain a registry of laboratories approved to hold and work with SARS CoV. Work involving culture, purification or concentration of live SARS CoV should be conducted in BSL-3 laboratories.

Closing ceremony

During the final session of the workshop, each of the three parallel sessions of 2 March were briefly summarized by a rapporteur. Dr Wang, Deputy Director, MoST, then outlined the most important outcomes of the meeting. Possible areas of collaboration between China and WHO were listed, including activities relevant to standardization of reagents and clinical trials. Examples of such collaboration could focus on the establishment of international repositories of SARS CoV isolates, standardization of SARS CoV antigen and antiserum preparation through a network of collaborating laboratories or support for the standardization of animal models. Main areas of collaboration in the clinical development domain could include capacity building in GCP, ethics and data management, production of a generic clinical trial protocols for the evaluation of vaccines against rare infectious diseases of high pandemic potential or review of clinical trial protocols.

On behalf of WHO, Dr Tarantola, Director, WHO Department of Immunization, Vaccines and Biologicals and Dr Kieny, Director, WHO Initiative on Vaccine Research, expressed their deep gratitude to the Government of China and the Chinese MoST for organizing and hosting such a productive and fruitful workshop. They were looking forward to the series of individual meetings planned with groups concerned with specific aspects of research on

SARS, H5N1 Avian Flu, HIV/AIDS, Japanese Encephalitis and Malaria. In addition, they would review the outcome of this meeting and other matters of common interest with officials in the Ministry of Health. These meetings would result in jointly agreed action plans building on the conclusions of the workshop.

ANNEX 1 List of Participants

CHINA

Invited Chairs, speakers and officials

Prof Yu Wang, Chinese Ministry of Science and Technology
Mr Jingdun Jia, Chinese Ministry of Science and Technology
Mr Yanfei Liu, Chinese Ministry of Health
Mr Wenzhuang Cao, Chinese State Food and Drug Administration
Mr Ju Jin, Chinese Ministry of Science and Technology
Prof Hongguang Wang, China National Center for Biotechnology Development
Dr Hongzhang Yin, Chinese State Food and Drug Administration
Dr Xiaoping Dong, Institute for Viral Disease Control and Prevention, China CDC
Dr Chuan Qin, Institute of Laboratory Animal Science, CAMS&PUMC
Dr Weidong Yin, Sinovac Biotech Co., Ltd.
Dr Huiying Shi, National Vaccine & Serum Institute
Dr Jiansan Zhang, Sinovac Biotech Co., Ltd.
Dr Bohua Liu, Chinese Academy of Military Medicine
Dr Yuanji Guo, Institute of Virology, China CDC
Dr Yuelong Shu, Institute of Virology, China CDC
Dr Yiming Shao, National Center for AIDS/STD Control and Prevention, China CDC
Dr Xiaofang Yu, Zhejiang University
Dr Wei Kong, Jilin University
Mr Zhe Yang, the Chinese Ministry of Science and Technology
Mr Sunan Jiang, the Chinese Ministry of Science and Technology
Mr Jijun Xin, the Chinese Ministry of Science and Technology

Invited experts

Prof Guowei Shang, Academician, Director General, National Institute for the Control of Pharmaceutical and Biological Products
Prof Yongxin Yu, Academician, National Institute for the Control of Pharmaceutical and Biological Products
Prof Kai Zhao, Former Director General, National Vaccine & Serum Institute
Prof Peitang Huang, Former Vice President, Chinese Academy of Military Medical Sciences
Prof Junzhi Wang, Deputy Director, National Institute for the Control of Pharmaceutical and Biological Products
Prof Jianguo Xu, Director General, National Institute for Communicable Disease Control and Prevention, China CDC

Invited participants

Dr Yongxiang Zhang, Chinese Academy of Military Medical Sciences
Dr Chao Liu, Chinese Academy of Military Medical Sciences
Dr Xuli Chen, Dept. of medical Sciences, Technology & Education, MoH
Prof Qinyu Zhu, Institute of Microbiology and Epidemiology, Chinese Academy of Military Medical Sciences
Dr Cuie Wang, Institute of Microbiology and Epidemiology, Chinese Academy of Military Medical Sciences
Mr Jiangting Chen, SinoVac. Biotech Co., Ltd.
Mr Qiang Gao, SinoVac. Biotech Co., Ltd.
Dr Jiahai Lu, The School of Public Health of Sun Yat-Sen University

Dr Yingqun Wang, SinoVac. Biotech Co., Ltd.
Dr Tao Ren, Huanan Agriculture University
Mr Tang Lin, Beijing Genomics Institute, CAS
Dr Songnian Hu, Beijing Genomics Institute, CAS
Prof Kejian Zhang, Center for Drug Evaluation, SFDA
Dr Enming Gao, Center for Drug Evaluation, SFDA
Dr Qingwen Meng, Harbin Veterinary Research institute of CAAS
Mr Hexiao Zhang, Beijing Entry-Exit Inspection and Quarantine Bureau
Dr Xiaoming Yang, China National Biotech. Corporation
Dr Aijun Hou, China National Biotech. Corporation
Dr Yongqiang Wu, China National Biotech. Corporation
Mr Xiaobo Liu, Department of Science, MoH
Dr Shumin Duan, Institute of Virology, China CDC
Dr Qi Li, Institute of Virology, China CDC
Mr Jinzhong Xiang, Dept. Drug registration, SFDA
Mr Jingqi Liu, Dept. Drug registration, SFDA
Mr Changgui Li, National Inst. for the Control of Pharmaceutical and Biological Products
Dr Guangmu Dong, National Inst. for the Control of Pharmaceutical and Biological Products
Prof Jie Chen, Peking Union Medical College Hospital
Prof Anping Ni, Peking Union Medical College Hospital
Prof Zhe Chen, Wuhan Institute of Virology, CAS

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Ms Yihong Hu, CNCBD
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Dr Zhenhang Yu, CNCBD
Mr Yuguo Zheng, CNCBD
Mr Naiyuan Zhou, CNCBD
Mr Guohui Han, Chinese Academy of Agriculture Sciences
Dr Fei Wang, Chinese Academy of Military Medical Sciences
Ms Xijuan Jiang, Chinese Academy of Military Medical Sciences
Ms Jin Wang, Institute of Virology, China CDC
Ms Yi Wang, Sinovac Biotech Co., Ltd.

WHO Guests

Dr Donna Ambrosino, Director and Professor, University of Massachusetts Medical School, USA
Dr Manon M.J.Cox, Chief Operating Officer, Protein Sciences Corporation, USA
Dr John David Clemens, Director, International Vaccine Institute, Republic of Korea
Dr Roland Dobbelaer, Head, Section for Biological Standardisation of the Scientific Institute of Public Health in Brussels, Belgium, Member of Expert Committee on Biological Standardisation (ECBS)
Dr Jacqueline Katz, Influenza Branch, CDC, USA
Dr Otfried Kistner, Director Virology, Baxter BioScience, Austria
Dr Ruth Macklin, Global Reference Group on HIV/AIDS and Human Rights, Vaccine Advisory Committee, WHO, USA

Dr Albert Osterhaus, Director, WHO Collaborating Centre for Arboviruses and Haemorrhagic Fever Reference and Research, Rotterdam, The Netherlands

Dr Punnee Pitisuttithum, Chief, Clinical Trial, Vaccine Trial Centre, Mahidol University, Thailand

Dr Jean-Francois Saluzzo, Aventis Pasteur, France

Dr Rebecca Sheets, National Institutes of Health, USA

Dr Zhiyi Xu, International Vaccine Institute, Republic of Korea

Dr Ping Yan, Health Canada, Canada

WHO Staff

Dr Henk Bekedam, Representative of WHO in China

Dr Marie-Louise R. Chang, Technical Officer, WHO-UNAIDS HIV Vaccine

Ms Nadia Fisher, WHO Geneva (assistant to Dr Kieny)

Dr Julie Hall, CSR Coordinator, WHO Representative Office in China

Dr Marie-Paule Kieny, Director, WHO Initiative for Vaccine Research

Dr Saladin Osmanov, Virologist/Immunologist, WHO-UNAIDS HIV Vaccine Initiative, Viral Vaccines, Initiative for Vaccine Research, WHO

Dr Daniel Tarantola, Senior Policy Adviser to the Director-General of WHO and Director, Immunization, Vaccines and Biologicals, WHO

Dr David Wood, Coordinator, Quality Assurance and Safety of Biologicals, Immunization, Vaccines and Biologicals, WHO

Mr Donald Zhang, WHO Representative Office in China

Ms Monica Zhou, WHO Representative Office in China