MEETING REPORT

WHO INFORMAL CONSULTATION ON CHARACTERIZATION AND QUALITY ASPECT OF VACCINES BASED ON LIVE VIRAL VECTORS

WHO HQ, Geneva, 4-5 December 2003

Contents

Summary

Live viral vectors provide an effective means for heterologous antigen expression *in vivo* and thus represent promising platforms for developing novel vaccines. While preclinical evaluation of live viral vectors has indicated their potential for immunization purposes, there is as yet limited clinical experience of their efficacy and safety. With increasing numbers of viral vectors now entering clinical trials, an urgent need to establish appropriate regulatory requirements to ensure their quality, safety and efficacy, has been recognized.

Key aspects of the development, production, quality control as well as nonclinical and clinical evaluation of vaccines based on live, viral vectors were considered in this global forum of regulators, vaccine developers, and academic experts. Discussion was focused on chimeric live flavivirus vectors, poxvirus vectors, adenovirus vectors, and alphavirus replicon vectors as candidate vaccines.

Current requirements for the production, control, nonclinical testing and clinical trials were presented by several National Regulatory Authorities (EU, USA, China, Canada) and issues for further considerations were recommended by the Consultation. Although several guidance documents provide general principles which apply to vaccines based on viral vectors, a need was identified for specific guidelines related to vectored vaccines.

Scientific issues of critical importance for further development of vectored vaccines were identified. Further investigations were recommended on the following issues: the potential for recombination of vectored vaccines with wild type pathogenic strains; implications of prior infections on the immunogenicity of vectored vaccines; the genetic stability of replicating vectors *in vitro* and *in vivo* and the potential for reversion to virulence; potential changes of vector tropism; and tests for replication competent virus in vaccine lots. In addition, a number of specific issues related to particular vectored vaccine are identified throughout the report.

Public acceptance of vectored vaccines was considered as a challenge for further development of these novel products and a need for a forum to discuss concerns and to communicate the views of leading experts in this area to the general public was identified. WHO was requested to coordinate the next steps to take this forward.

The Consultation recommended that WHO publishes the detailed meeting report as a first step towards the development of the WHO guidelines. This meeting report has been presented to the Expert Committee on Biological Standardization at its meeting in 2004 to obtain advice on the way forward.

Introduction

Beyond conventional vaccines which include live attenuated, inactivated, toxoid, subunit and conjugated vaccines much progress has been made towards the development of novel vaccines and vaccination approaches. It is recognized that effective *in vivo* antigen production, achieved by using modified live viruses as vectors to deliver antigen(s) or antigen-encoding gene(s) to vaccination sites, is a promising approach for future immunization. For example, much relevant scientific and clinical data concerning the design, manufacture, non-clinical testing, and safety of recombinant vaccinia virus vaccines has accumulated over the past 20 years. Such data indicate the potential "strengths and weaknesses" of viral vaccines that express heterologous antigens. Live viruses considered for development encompass attenuated, replication restricted, and replication defective vaccine candidates.
The WHO has recognized that novel technologies involved in the development and manufacture of live viral vectors will generate new quality, efficacy and safety issues. To review experiences with the development and evaluation of vaccines based on live viral vectors and to discuss related regulatory issues, a Consultation, jointly organized by the WHO "Initiative for Vaccine Research" and the "Quality Assurance and Safety of Biologicals" team, was held in Geneva, 4-5 December 2003. The Consultation was attended by representatives from National Regulatory Authorities (NRAs), the vaccine industry and academia (list of participants in annex 1) and chaired by Dr Klaus Cichutek (Paul Ehrlich Institute, Germany); Dr Anthony Meager (NIBSC, UK) served as rapporteur.

Meeting Objectives

The objectives of the consultation were to:

i) Review the experience with development and evaluation of vaccines based on live viral vectors: adenoviruses, poxviruses;
ii) Discuss needs for standardization and control of live viral vector vaccines, and
iii) Consider regulatory concerns and requirements in this area.

The expected outcomes of the Consultation included i) better understanding of requirements for developing appropriate quality, efficacy and safety testing for full characterization of vaccines based on live viral vectors, ii) mapping out requirements for their clinical trial approval and licensure, and iii) identifying needs for the development and establishment of appropriate reference materials to improve quality control, as well as efficacy and safety testing.

Scope of the Consultation

It was agreed that the following areas should provide the main focus for discussion:

i) Vectored vaccines derived from attenuated viral vaccines (i.e., chimeric live vaccines based on attenuated Yellow fever virus - YFV),

ii) Vaccines based on replication-restricted-poxviruses (i.e., avian poxviruses, attenuated vaccinia virus strains), -adenoviruses, parvoviruses (i.e. adeno-associated viruses - AAV) and -herpesviruses expressing heterologous antigen-encoding genes, possibly in combination with immune stimulator-encoding genes,

iii) Vaccines based on alphavirus replicon vector particles expressing heterologous antigen-encoding genes (e.g., replication-defective viral replicon particles derived from Semliki Forest virus (SFV), Venezuelan equine encephalitis virus (VEE) and Sindbis virus (SIN)) ,

iv) Prime-boost vaccination approaches involving the sequential administration of vaccine types itemized in i) to iii).

The development of such vaccines is primarily for prophylactic use against infectious diseases, but they can also be considered for use as post-infection or therapeutic vaccines against infectious diseases and tumours.

Other vectors, including virus-like particle (VLP) vaccines and retroviral carrier-based vaccines with antigen display structures (e.g. murine leukemia virus {MuLV}-display vaccines) and those based on prokaryotic cells (e.g. *Salmonella typhimurium*) or eukarytotic cells (e.g. dendritic cells) were considered out of scope for the Consultation.

1. Characterization of vaccine candidates and nonclinical testing

1.1. Chimeric live flavivirus vaccines (Dr. T. Pullano, Acambis, Inc.)

Dengue virus (DE), Japanese encephalitis virus (JE) and West Nile virus (WN) are three flaviviruses which can cause serious illness and for which development of safe, effective vaccines is a priority. Chimeric live viral vaccines based on the 17D strain of YFV have been produced for immunization against DE, JE and WN by replacement of 17D viral envelope (*E*) protein genes with those of DE, JE and WN, respectively. Attenuating mutations are also engineered into the *E* genes of JE and WN. Chimeric viruses are propagated in Vero cells grown on microcarriers in bioreactors and isolated from supernatant harvests by ultrafiltration and sterile filtration steps. When tested *in vitro* 100% of plaques express heterologous viral envelope genes. Although their replication *in vivo* is limited, they apparently induce protective, durable immunity by stimulating the

development of neutralizing antibodies specific for the heterologous envelope proteins with only a single dose.

Genetic stability and absence of neurovirulence are crucial to the safety of chimeric live flavivirus vaccines. Since flaviviruses lack proof-reading enzymes for replicating their ssRNA genomes, point mutations accumulate during propagation. It is of utmost importance therefore that the attenuated phenotype of seed chimeric viruses and vaccine lots are retained. Sequencing of viral RNA is not however sensitive enough to detect reversion to less attenuated variants and thus virus seeds and vaccine lots are subjected to mouse and monkey neurovirulence tests. Experience at Acambis showed that the 17D strain of YF used as platform for chimeric vaccine gives detectable, but very low levels of neurovirulence in these tests. Chimeric virus, in contrast, appear to have a lower level of neurovirulence in the same tests.

JE/17D, DE (single serotype)/17D and WN/17D chimeric viral vaccine products are currently in phase IIB (JE) and phase I (DE, WN) clinical trials. Both have been shown to be safe and well-tolerated, yielded only low viremias, and induced development of neutralizing antibodies in 100% of vaccinated subjects with no apparent interference by prior YFV vaccination. Chimeric vaccines based on YFV are new viruses, which give rise to concerns that they may replicate and spread as a new pathogens to the environment. However, only low viremias have been detected after administration. In addition, only a few mutations have been detected in progeny virions. Although determinants of transmissibility are difficult to identify, these initial data suggest further careful evaluation of the chimeric vaccine candidates is warranted.

There was general consensus to establish a clear scientific rationale for carrying out neurovirulence testing in non-human primates. Detection of mutations known to be associated with reversion to less attenuated variants should obviate the need for neurovirulence tests, but current information indicates that mutants representing less than 10% of the total population cannot be detected by sequencing. Thus, a requirement for *in vivo* virulence testing remains. It was agreed that a homologous reference preparation to study batch to batch reproductibility would provide a better comparator. However, since DE envelope proteins show no tropism for brain cells, the necessity to test the DE/17D chimeric vaccine for neurovirulence was considered debatable.

Conclusions: Chimeric live flavivirus vaccines

- The level of attenuation should be precisely assessed.
- Sequencing and exclusion of mutations mediating reversion of the attenuated phenotype to virulence may be used as a test for production characterization.
- Chimeric live virus variants encompassing genes from different viruses are new human viruses if the antigen gene (e.g., viral envelope) is involved in replication and may have effects on tropism.
- A clear scientific basis for neurovirulence testing in non-human primates should be given. Neurovirulence in mice may be used to show seed-to-batch and batch-

to-batch consistency as well as level of attenuation compared to a relevant reference virus.

1.2. Vaccines based on live recombinant viral vectors encoding and expressing heterologous antigens

1.2.1. Vaccines based on poxvirus vectors (Dr. G. Sutter, Paul Ehrlich Institute; Dr L. Mallet, Aventis Pasteur; Dr J.M. Balloul, Transgene; F. Verdier, Aventis)

Among large DNA viruses, poxviruses, and in particular vaccinia virus and avian poxviruses (canarypox and fowlpox), have long been consideredas promising candidates for the development of vaccines for use in humans and in the veterinary field.

Advantages of poxviruses for vaccine development include:-

i) relative ease of generation of recombinant viruses
ii) high/large capacity for insertion of large amounts of heterologous DNA
iii) cytoplasmic replication and no evidence for genomic integration of viral DNA, nor for persistence and oncogenic potential
iv) gene expression is regulated by virus-specific promoters and enzymes
v) gene expression can be obtained in a wide range of host cells
vi) for the avian poxviruses, restricted ability to replicate in human cells

Characteristics of the candidates: Attenuated strains of vaccinia virus (e.g. MVA, NYVAC), fowlpox virus (e.g. FP9, TROVAC) and canarypox virus (e.g. ALVAC), which replicate in chicken embryo fibroblasts (CEF), but not in human cells, are widely used for the development of vectored vaccines.

Characteristics and Production of MVA

For modified vaccinia virus Ankara (MVA), an attenuated strain with established clinical safety (Drexler et al., Curr. Opin. Biotechnol. 2004), specific methods are available for engineering and selecting recombinant viruses containing foreign (antigen) genes. Nucleic acid amplification technology (NAT) based assays have likewise been developed for monitoring genetic homogeneity and gene transcription. In addition, technologies using antigen-specific antibodies have been developed to enable antigen expression to be monitored, e.g., by Western immunoblotting, or by immunostaining of recombinant MVA virus-infected cells *in vitro*. Recombinant MVA viruses encoding various antigens of disease causing viruses, e.g. influenza virus, parainfluenza virus, measles virus, respiratory syncytial virus, dengue virus, Japanese encephalitis virus, HIV-1, have been characterized *in vitro* and tested as vaccines in *in vivo* model systems for immune stimulatory effects, e.g. development of antigen-specific T cells and antibodies. Despite abortive replication in human cells, the heterologous genes are expressed and induce immune responses. Infected human cells succumb to apoptosis and are cleared by immune-mediated mechanisms.

As with other live virus vectors, recombinant MVA can be propagated at large scale and it is necessary to ensure a suitable level of operating safety containment to protect personnel and prevent dissemination to the environment.

It was commented that highly useful host range selection methodologies for generation of recombinant MVA (e.g., based on viral E3L or K1L gene expression) could be possibly constrained by the lack of certified cell stocks of RK13 (rabbit kidney cell line) or BHK-21 (baby hamster kidney cell line) cells. Moreover, these cell lines have not previously been approved for production of human vaccines.

Characteristics, production and quality control of poxvirus vectors

Traceability from plaque isolation to production of Master Virus Seed, control of the production process to ensure genetic stability at all stages of manufacture, and development of QC assays to fully characterize recombinant products are essential. A full range of QC assays for assessing sterility, absence of contamination by mycoplasmas, extraneous agents, cellular DNA and protein, animal proteins (e.g. trypsin), antibiotics, identity, infectivity and biological activity should be applied to ensure that the bulk- and final product-specifications fall within acceptable limits. For example, total DNA content is comprised of DNA from mature ALVAC particles, damaged/immature particles, replication complexes and cellular DNA for which determination of total DNA is carried out by a picogreen-based assay, viral DNA by quantitative polymerase chain reaction (qPCR), and mature particle DNA by qPCR after DNAase digestion. Total protein is determined by the Bradford method. Infectious titre is estimated by CCID50 assay on QT cells. Regarding adventitious agent testing of recombinant MVA and ALVAC batches, it was highligthed that it is difficult to develop a compliant assay, as reagents (i.e. neutralizing antibodies) to fully neutralize poxviruses are not available.

Biological activity is usually measured in potency assays. Data generated in studies on vector biology, presented in this section (below), have been considered useful for further development of such potency assays. However, at this stage they cannot be interpreted as formal potency tests for the viral vectored vaccines in question.

Since mutations and deletions within MVA or ALVAC vector and inserted construct DNA can occur at high passage levels, there was a need to monitor consistency of antigen expression to ensure efficient and stable expression of the inserted antigen gene(s). The development of validated potency assays to determine expression levels is thus required together with appropriate reference materials to permit comparison of results across assays and batches. For ALVAC-HIV, for example, potency assays have been based on i) HIV-1 gene expression in CEF (immunoplaque assay); ii) HIV-1 gene expression in human HeLa cells (FACS analysis); iii) immunoreactivity of HIV-1 p24 and gp120 following lysis of infected HeLa cells and iv) cellular immunogenicity following immunization of mice (IFN-γ ELISPOT assay). The immunoplaque assay *using* double staining of expression product (antigen) and ALVAC vector allows to demonstrate a

consistent ratio between antigen and vector. Results so far obtained for the master seed indicated virtually 100% of infected CEF expressed heterologous antigen genes. However, a specification for the antigen expression level (if less than 100%) attained after passaging would be useful In contrast, consistently only 70% of HeLa cells expressed antigen following infection. The less than expected expression level may be due to limited amounts of HIV antigen made upon early gene expression in human HeLa cells, in which replication of ALVAC is abortive. There is still a need to determine the variability of such tests and if possible to achieve valid comparability of the results from different assays.

It was acknowledged that not enough is known about the immunogenic properties of poxvirus vectors. For instance, the *in vivo* roles of their immunoregulatory genes are far from being understood; how for example are vaccine properties influenced, and will it be possible to derive further improved vector backbone viruses? Furthermore, there is little information on how the number of antigen gene insertions in recombinant poxviruses affects genetic stability and growth capacity of the vector product. Regarding antigen expression, it remains unclear how important correct post-translational modification, e.g. glycosylation, is for antigen presentation.

Nonclinical testing

Nonclinical safety testing, *in vivo,* is assessed in experimental models to evaluate virulence and acute toxicity. In mice, inoculated intracerebrally or intraperitoneally, ALVAC and recombinant ALVAC vaccines, e.g. ALVAC-HIV, were shown to be less virulent than other vaccinia virus strains tested. In mice and rats, no signs of acute toxicity were found for ALVAC vector/vaccines. Biodistribution studies, including a platform study of the ALVAC parental vector, were carried out in rats and monkeys using subcutaneous (*sc*), intramuscular (*im*) and intravenous (*iv*) routes of administration; a PCR method was used to detect viral DNA locations in necropsy organ and tissue samples. *Subcutaneaous* administration in rats of ALVAC vector led only to local but persistent detection of vector DNA at the injection sites; *im* administration led to peak DNA levels in muscle at day 7 post-injection, but DNA was undetectable at day 45; *iv* administration led to initial wide-spread distribution to all tested organs, including gonads and brain, but viral DNA was not detectable after 7 days post injection. Repeated *im* administration of ALVAC-HIV led to viral DNA being detected at injection sites, but all organs/tissues tested after 1 day, or a 2 weeks recovery period, were negative. A study in monkeys to investigate whether ALVAC-HIV increases sensitivity to HIV infection, due to potential development of “enhancing antibodies”, has been conducted. A repeated dose toxicology study with ALVAC-HIV has also been carried out in monkeys, but no toxicity was observed. Little or no shedding of ALVAC-HIV was found at injection sites and none was found in body fluids suggesting that the risk of spread to the environment was low.

1.2.2. Vaccines based on adenovirus vectors (Dr. B. Ledwith, Merck Research Laboratories)

Characteristics, production and quality control

Adenoviruses, of which there are many human and animal serotypes, are large DNA viruses. Human adenoviruses are associated with mild respiratory infections; most of the population has some antibodies against them. Adenoviral DNA is linear, double-stranded DNA of 36 kb divided into several early (E) and late (L) gene regions. Foreign genes may be readily incorporated into adenoviruses by replacement of the viral E1 region with intended transgene construct. The resulting recombinant adenoviral vectors are replication-deficient in target cells, but can be propagated in cells with integrated complementing E1 genes expressed *in trans*. Adenoviral vectors of this kind based mainly on human adenovirus type 5 (Ad5) have been mostly used for *in vivo* gene therapies of monogenic disorders, malignancies, and cardiovascular disease. However, they are increasingly considered for the development of Ad vectored vaccines. For example, plaque-purified isolates of Ad5 engineered to express consensus genes encoding HIV-1 clade B *gag* or trivalent *gag/pol/nef* may be propagated in E1-complementing PER.C6 cells. Such Ad5 vector preparations are characterized with respect to i) identity by PCR and RFLP (Southern blot), ii) concentration (particle number) by Q-PCR, iii) potency by $TCID_{50}$ and Q-PCR (for infectious units) and *in vitro* gene expression, iv) purity by measuring cellular DNA, RNA and protein plus process residuals, and v) other aspects including stability, sterility, absence of adventitious agents and replication competent adenovirus (RCA). The manufacturing process yields highly purified vector particle preparations containing >95% Ad5 protein, <30 pg cellular DNA/10^{11} virus genomes and extremely low levels of process residuals.

Nonclinical testing

Immunogenicity of Ad5-vectored HIV-1 vaccines has been studied in mice and rhesus monkeys, in which they elicited strong cellular immune responses. An IFN-γ ELISPOT assay together with flow cytometry and CTL cytotoxicity assays is used to measure cellular immune responses. These vaccines could also be shown to induce antibody responses. In a monkey challenge model Ad5 SIV gag vaccine was shown to protect against SHIV-induced AIDS. From a toxicological point of view, such Ad5 vectors can be considered as similar to attenuated live viral vaccines. Local tolerance studies were carried out in rabbits and systemic toxicity studies in monkeys and mice, with a prospective move to rats to enable full human doses to be administered. The vaccines were well-tolerated up to doses of 10^{11} particles/dose with results of toxicity testing consistent with the expected immune responses to the Ad5 vector, e.g. local irritation at site of injection, lymph node and spleen enlargement, slight transient changes in hematology parameters. Only one example of liver toxicity was recorded in a mouse injected with Ad5 trivalent gag/pol/nef vaccine. Appropriate developmental and reproductive toxicity studies are to be performed prior to licensure as vaccine will be administered to women of child-bearing age. Biodistribution studies of Ad5 vectored

vaccines using PCR methods to detect vector DNA showed that, following *im* injection, the vector persisted mainly near the injection site and in draining lymph nodes for up to 6 months. Vector DNA levels decreased with time as did also transgene expression levels. The vast majority of the vector DNA remained at extrachromosomal locations, but, using a sensitive "repeat-anchored integration capture" (RAIC) assay, a low level of integration into chromosomal DNA was observed. Up to 200 copies (10 fg) of vector DNA per µg chromosomal DNA may be integrated. On the assumption that all vector DNA copies are integrated into host cell genes causing their inactivation, this would lead to a mutation rate of 2×10^{-7} mutations per cell, a worst case scenario, which however is 10-fold less than the spontaneous mutation rate of 2×10^{-6} mutations per cell.

In summary, non-clinical safety studies in animals (mice, rabbits, monkeys) of Ad5-vectored HIV-1 vaccines have shown them to be well-tolerated and safe, even above the projected clinical dose, and to induce the development of relevant immune responses. Biodistribution studies have shown most vector DNA remains in tissues close to the injection site and have indicated a low rate of insertional mutagenesis.

In discussion questions were raised regarding the level of Ad5 vector DNA integration into chromosomal DNA. It was perceived that further studies were needed to clarify whether integrations were random, were or were not in active genes and whether there were any favoured "hotspots" for insertions. It remains controversial whether integrating viruses should be used at all as preventative vaccines.

1.3 Alphavirus replicon vectors as vaccines (Dr. J. Polo, Chiron Corporation)

Characteristics and production

Alphavirus replicon vectors based on the RNA viruses SFV, SIN and VEE are proposed as preventative vaccines, but are not as advanced in their development as attenuated poxvirus vectors or adenovirus vectors. They are produced by replacing the alphavirus structural protein genes with the transgene construct, thereby rendering the vectors replication or "propagation" incompetent. The RNA replicons may be converted into functional into functional DNA formats that directly transcribe the RNA replicons *in situ,* or the RNA replicon may be packaged directly into virus-like particles. The physical properties and production of plasmid DNA replicons are generally similar to "conventional" plasmid DNA vaccines. Alphavirus replicon vector particles can be produced either by co-transfection of in vitro transcribed "helper" RNA containing the alphaviral structural protein genes or (better) by transfection of stable packaging cell lines containing inducible alphaviral structural protein gene cassettes to enable replicon RNA to be packaged. Replicon particles can be readily purified.

Nonclinical testing

Multiple publications by several groups have demonstrated immunogenicity for each of the alphavirus replicon particle platforms (e.g., SIN, SFV, VEE) in a variety of animal models. SIN replicon vector particles expressing several different antigens, such as HIV-1 antigens, gag or env, or HSV-gB antigen have been shown to induce specific cellular and humoral responses in mice following administration by *im* or intranasal routes. Chimeric (VEE/SINV) replicon vectors encoding HIV gp140 have been shown induce specific immune responses in prime-boost regimens in rabbits that exceed those obtained with adjuvanted HIV gp140 protein alone. Rhesus monkeys immunized with SIN HIV gp140 replicon particles were primed well for a second HIV gp140 protein boost and developed HIV env-specific antibodies. Good immune responses against influenza hemagglutinin (HA), with an advantageous shift to Th1 responses in comparison to the Th2 response induced by HA alone, were obtained following immunization with SIN-HA replicon particles in mice.

In summary, alphavirus replicon vector particles constitute potentially interesting vaccine delivery platforms. Several advantages are perceived and include i) cytoplasmic RNA-based expression which precludes the possibility of genome integration ii) induction of the immune response in animal models (e.g., mice, rabbits, nonhuman primates) iii) induction of broad humoral and cellular immune responses, iv) intranasal (mucosal) delivery, and Iv) well-tolerated and safe. Clinical data for both plasmid and particle-based replicon vaccines should begin to be available in the next year.

In discussion, it was commented that serum responses to replicon vaccines seemed short-lived, especially to HIV gp140. It was argued that this could be "antigen-specific" response.
It was pointed out that, in theory, non-homologous recombination can happen leading to the generation of replication competent virus, but propagation systems are designed to prevent this occurring; at least two recombination events are required, but high titers (10^{12}) may be used, which increase risk of recombination. Repeat dosing would also increase the risk of recombination. The possibility of superinfection with wild type viruses was mentioned, but testing to rule out recombination events was difficult to undertake with replicon particles. In the specific case of the influenza HA replicon vaccine it was suggested that neutralizing antibody data rather than data from other tests, e.g. inhibition of hemagglutination, would be of more relevance for determining efficacy.

1.4 General conclusions

Production and quality control of vaccines based on live, viral vectors

- As with all vaccines, the method of production should be well described and parameters for manufacturing consistency established. Traceability of all components should be ensured, including cell bank, virus master seeds, vector

master seeds and the development of genetics of vector expression and stability of the construct should be required.

- General principles for use of cell substrates for production of biologicals should be followed (4). Further consideration will be needed on a case-by-case basis, particularly if cells used have not previously been used for vaccine manufacture. Well-characterized master cell bank and pure virus/vector seed bank needed; high level of correct sequence and absence of adventitious agents including viruses derived from vector needed at this stage. Stability of packaging cells used, if applicable, should be ensured.
- Plaque purification, if applicable, or equivalent methods are relevant.
- Potency assay should be clearly defined. Generic reference material, e.g. vector without transgene, may be useful for potency assays to show assay comparability between different laboratories´ assays; biological or physical assays may be considered and should be appropriate to the intended purpose One or more potency assays should be used to ensure batch-to-batch consistency.
- Stability of the construct: assessed by showing consistency of production, monitoring mutations and inserts at post-production passage levels. Genome stability should be assessed as discussed below.
- Genome stability may be addressed by assessing the percentage of *in vitro* infected cells expressing the transgene during master/working seed banking (or master/working cell banking if packaging cell line used), during manufacture and for batch release testing. The percentage observed should remain stable. The absolute number depends on the sensitivity of the assays used for intracellular expression of the vector proteins versus that of the assay used to measure foreign gene product expression. There is a need for some guidance on the assessment of genetic stability (e.g., appropriate passage levels).
- Purity: may be difficult to detect or remove extraneous material such as DNA, cells, cell protein etc. If they cannot be completely removed, the limits should be set as appropriate.
- The total contaminating protein content should be kept to a minimum in order to avoid development of adverse host immune reactions.
- Foreign nucleic acid contamination, e.g. from packaging cell, should be kept to a minimum.
- Sequencing of foreign gene insert plus a small part of the flanking regions is necessary to assure identity of the foreign gene inserted and of the vectored vaccine recombinant.

Non-clinical testing of vaccines based on live, viral vectors

Non-clinical safety evaluation of vectored vaccines includes separate assessments of i) the vector platform itself, ii) the safety of the vector expressing the vaccine antigens, and iii) the vectored vaccine with adjuvant (if appropriate).

It was noted that current Regulatory Guidance documents are, in principle, helpful for planning non-clinical testing strategies. WHO guidelines on nonclinical evaluation of

vaccines is available and offers principles for nonclinical testing of vaccines, in general, as well as some guidance for viral vectors (1,3,5). However, current guidance do not clarify whether vector "generic" (or platform) studies or construct specific studies are acceptable/most appropriate and, if platform studies are appropriate, which studies need repeating for the construct-containing product. Where only small changes in construct composition are made, less repetition of testing may be possible. Guidance is also lacking on what to do if, for example, ALVAC vaccines are combined with other products; the perception was that new studies would be required. It was agreed that the need for additional studies with ALVAC-construct vaccines should be considered in relation to the target population.

The consensus opinion was that animal testing following appropriate guidelines was required, but that testing in non-human primates may not always be essential; where possible, alternatives should be considered. However, for any new vaccine safety testing, but not immunogenicity testing, in non-human primates was agreed to be essential. The dose to be used in animal tests should be comparable to the highest dose used in phase I clinical trials. It was recognized however that volume may be a limitation on how much vaccine can be administered to very small animals, such as mice, and that where possible larger animals, e.g. rats, rabbits, should be considered for tests of vaccine doses equivalent to human doses. Immunogenicity studies should include those to check for the development of enhancing antibodies. It was recognized that guidance on what to do in the case of unexpected results, e.g. kidney toxicity with ALVAC + GM-CSF, was currently lacking. It was however expected that such results should be included in the full benefit: risk assessment. Guidance was also lacking on the extent of new studies that should be performed on recombinant viral vaccines, i.e. vector plus transgene, following platform studies with the vector alone.

There was general concern for vectored viral vaccines that retained some capacity to integrate their DNA into chromosomal DNA. This had been highlighted by the finding that recombinant Ad vaccines integrate their DNA at a low, but significant, level. The consensus opinion was that more studies, particularly those aimed at identification of integration sites, would be required for vectored viral vaccines whose DNA entered the cell nucleus; such studies were felt not to be necessary for vaccines, e.g. recombinant poxviruses, where there was no evidence of a nuclear phase. It was argued that any evidence found for insertional oncogenesis occurring that led to increased risk of cancer development would be unacceptable for a vectored viral vaccine. The fact that in rats which received 10^{11} Ad particles (human dose), showed no tumour development was however reassuring, suggesting that the risk of insertional oncogenesis with recombinant Ad vaccines is likely to be very small. Nevertheless, clinical experience with Ad vaccines is limited and therefore tumorigenicity studies should continue to be part of their risk assessment.

Non-clinical testing

The following aspects need to be addressed during vectored vaccine development

    - Non-clinical platform safety studies of the vector itself, may be useful for several products based on the same vector
    - Non-clinical safety of proteins expressed by specific vectored vaccine
    - Non-clinical safety of specific vectored vaccine
    - Non-clinical safety of prime-boost approaches

  Test acute and repeated dose toxicity, the latter depending on the human vaccination schedule planned.
- Platform studies with vector alone may be of value to address general vector issues.
- Novel vectors not having been previously used in humans should be tested in non-human primates to address safety concerns, if appropriate**.**
- Immunogenicity, toxicity, biodistribution, genotoxicity (but not reproductive/developmental toxicity) as appropriate should be assessed before phase I
- Developmental toxicity for a vaccine to be used in adult women of child-bearing age should be assessed after phase I.
- No general animal model is to be recommended.
- Maximum tolerated dose (MTD) testing is not necessary.
- Evaluation of genetic reversion also to be done *in vivo*.
- Unexpected events in animal studies: any unexpected adverse event observed in a non-clinical study should be further addressed, but does not always constitute a no-go signal for further development or clinical testing.
- Biodistribution:
    - monitor persistence at injection site,
    - biodistribution,
- Integration of viral nucleic acid where uptake into nucleus is possible.
- Acceptability for preventive vaccines would depend on a quantitative risk assessment. The rate of spontaneous gene mutation may be considered as a reference bench-mark. With preventive vaccines which have been found to lead to vector integration, integration site analysis may be useful to further characterize the potential for mechanisms leading to oncogene activation or tumour suppressor gene inactivation. An alternative may be the application of a sensitive viral carcinogenicity animal model.
- Carcinogenicity studies may be required
    - e.g. for human Ad shown to have increased oncogenicity in animals or
    - or for uncharacterized simian Ad.
    - to test cell substrates
- Integration assays are considered appropriate for vaccines able to deliver nucleic acids into the nucleus: Ad, AAV and, DNA with replicons, but not for poxvirus- and alphavirus-based vectors .

2. Reference materials and standardization

(A. Meager, NIBSC)

A range of viruses, including adenoviruses, parvoviruses (adeno-associated virus), poxviruses (vaccinia, canarypox), positive-strand RNA viruses (SFV, SINV, VEE, YFV, poliovirus, Kunjin virus), negative-strand RNA viruses (influenza virus, Sendai virus) and double-strand RNA viruses (rabies virus, vesicular stomatitis virus, retroviruses) have been considered for the development of vectored viral vaccines. This intended use raises a number of standardization issues, including comparability of results of infectivity and potency assays among laboratories, dose definition of product and what reference materials are appropriate for assay calibration and sensitivity monitoring. These issues relate not only to quality and efficacy, but also to safety. For some viruses, determination of virus particle number is paramount in assuring safety, e.g. for adenovirus, which is markedly toxic at high dose levels, while for others this may be less important. For instance, infectivity and antigenicity as measured in functional assays, are for many viruses important characteristics for determining safe, efficacious doses when used as vaccines. However, currently there are very few reference materials universally available for standardization purposes. Only in the area of Ad vectors has there been concerted international action to produce and characterize a suitable reference material.

The unexpected death of a young patient with ornithine transcarbamylase (OTC) deficiency following acute systemic toxicity, as a direct result of administration of an Ad5 vector, prompted much greater regulatory oversight on the quality and safety of adenoviral vectors. Many analytical tests, e.g. to determine Ad particle numbers by a variety of methods, including UV absorbance measurement of viral DNA, HPLC of viral proteins, picogreen binding to viral DNA, and qPCR, were being used, but generally there was a lack of standardization, thus making difficult comparisons of test results from different manufacturers. Also, when data are generated from tests performed without appropriate reference materials, confidence in safety assessment is usually lacking. To address this issue, a wild type Ad5 reference material (ARM) has been prepared and characterized with respect to particle numbers and infectious units. The work to select, prepare and characterize the ARM was carried out by members of an *ad hoc* ARM Working Group (ARMWG), set up in 2000, with collaboration of CBER, FDA (USA). Statistical analysis of data from a collaborative study, undertaken by ARMWG members, for determining particle numbers, infectivity and stability of the ARM led to assignment of 5.8×10^{11} particles/ml and 7.0×10^{10} infectious units/ml to the ARM. Long-term stability studies are on-going; they indicate that the ARM is completely stable at -80 C but will lose both particle concentration and infectivity if stored at -20 C or above. Vials of the ARM (Cat. No. VR-1516) containing 0.5 ml are stored frozen at -80 C at the ATCC Repository in the US (ATCC: The Global Bioresource Center). From August 2002, they have been available to investigators wishing to establish the particle concentration and infectious titre of their own Ad vector preparation. The latter can then be used as an internal (working) reference material to determine the values for preparations with unknown particle concentration and infectious titre. Between August 2002 and October 2003, ATCC has shipped 261 vials of the ARM to 12 countries.

Problems with dispatch and use so far notified include losses of particle concentration and infectious titre due to dry-ice sublimation and exposure to carbon dioxide during transit to far destinations, instability once thawed due to particle aggregation and lack of adherence to proper usage of the ARM; these should be readily overcome. However, it remains unclear whether the ARM will be suitable for other Ad serotypes, e.g. Ad2, or for Ad vectors with advanced design features, e.g. those with modified capsids and high capacity (gutless) Ad vectors. There is a perceived need to carry out comparative studies. The ARMWG is recommending that investigators send back data to enable review of its usefulness to be done.

Besides the ARM, work is about to begin on the preparation and characterization of an adeno-associated virus reference material (AAVRM). This is to be undertaken by a newly set up AAV Reference Standard Working Group (AAVRSWG) and will proceed in a manner styled on that of the ARMWG. In addition, a Working Group on considerations for the preparation of lentiviral vector reference materials has been established, but has not yet made recommendations due to the wide diversity of lentiviral vectors being developed.

In discussion concern was expressed that in the development of new gene transfer reference materials, e.g. the ARM, the WHO had not been involved or consulted. It was agreed that in future the WHO should be involved as its expert advisors could provide specific advice on the preparation of such reference materials. It would also be of value to establish reference materials as WHO international standards to ensure universality of their use.

Although counting particles is possible for several viruses, it was debatable how useful this was for live virus-vectored vaccines. In some cases, e.g., enveloped viruses, particle enumeration remains difficult. It was felt that potency determinations may be more relevant for viruses other than Ads. A reference standard for MVA calibrated for IU could be useful for QC testing and ensuring consistency of production.

Reference materials: conclusions

- Ad5 reference material (ARM) was developed and made available internationally. It was needed to standardise Ad particle number in systemic clinical use of Ad vectors to avoid acute toxicity.
- ARM is stable and useful for the determination of Ad particle number; may also be useful to standardise Ad infectivity assays.
- Reference material preparations should be prepared according to GMP. WHO expertise would be useful during development of new reference materials.
- ARM may be useful for particle measurements in replication-incompetent adenoviral vectored vaccine, e.g. to relate local reactions to the particle dose applied.
- MVA reference material may be useful for *in-vitro* infectivity assays.
- Reference materials for other live viral vectors should also be developed and available at licensing stage.

3. Review of vectored vaccines in clinical trials

(Dr S. Gurunathan, Aventis Pasteur; P. Pitisuttithum, Mahidol University, Thailand)

3.1. Recombinant ALVAC and MVA vaccines

Immunogenicity testing of ALVAC vaccines has shown that they elicit antigen specific immune responses, including development of neutralizing antibodies, antigen-dependent cell-mediated cytotoxicity (ADCC), lymphoproliferative and CD8+ CTL responses. Compared to attenuated vaccinia vaccines, ALVAC vaccines also have the advantage that their efficacy will not be affected by pre-existing immunity to vaccinia. Currently there are several ALVAC vaccines licensed for veterinary use, e.g., in canaries, horses, dogs and cats with no safety concerns. In addition, several ALVAC-antigen vaccines have been developed that have been or are being clinically evaluated. Many clinical trials have already been conducted in volunteers (over 1,800) with ALVAC-HIV candidate prophylactic vaccines. The latter usually are engineered to express multiple HIV antigens, e.g. gp120, gag, pro (vCP205, vCP1521). No dose response for CTL could be demonstrated with ALVAC-HIV, but repeated immunizations increased the CTL responses.

A first phase III prime-boost study in 16, 000 volunteers (Thailand) involving priming with recombinant ALVAC- HIV (vCP1521) vaccine and boosting with HIV-1 gp120 B/E clade protein has recently started. A phase I study comparing the safety, tolerability and immunogenicity of Ad5 HIV-1 gag and ALVAC-HIV-1 (vCP205) in volunteers who previously received only the Ad5 HIV gag vaccine is also in progress. In addition ALVAC-HIV-1 is being tested as a therapeutic vaccine in more than 400 HIV-1 seropositive volunteers. Therapeutic ALVAC vaccines, used alone or in combination with cytokines, tumour antigens, or chemotherapeutic agents, against melanoma and colorectal cancer are also in phase I clinical trials. Some positive anti-tumour responses or disease stabilizations have been observed so far.

Regarding ALVAC vaccine safety, data so far accrued has been consistent across constructs and populations. Reactogenicity, probably mainly due to the vector backbone, has involved mild reactions, e.g. pain, erythema, at the site of injection. Systemic reactions have also been mild, e.g. low fever, and nothing remarkable concerning adverse events has been recorded. Furthermore no safety concerns have been generated with the use of ALVAC in HIV + individuals. In terms of environmental safety and possible dissemination of ALVAC vaccine as a GMO, shedding studies have proved that all blood and secretion samples tested negative indicating a very low risk of dissemination.

MVA-based vaccines are also being evaluated in prime-boost vaccination regimens in combination with other vaccines including heterologous live viral vectors. Early data from clinical trials demonstrating the immunogenicity of MVA vector vaccines in

humans are now available. These include results from immunizations of healthy volunteers with recombinant MVA expressing genes encoding HIV or malaria antigens and from immunotherapeutic vaccinations of HIV-1-infected individuals with recombinant MVA encoding HIV-1 Nef as antigen.

It was recognized that pre-existing immunity to vaccinia virus could potentially reduce the efficacy of recombinant MVA vaccines. However, it has been shown that priming and amplification of immune responses specific for new antigens expressed by recombinant MVA can be achieved. While replication-defective MVA can be used to induce an immune response at least similar to standard vaccinia virus, it was acknowledged that further comparison of the effectiveness of recombinant MVA with other poxvirus strains is desirable. For example, while the genetic bases and typical phenotypic properties of MVA and NYVAC are known, it remains unclear as to which is superior for vaccine development. While MVA vectors have a good safety record, it was shown upon vaccination with replication competent vaccinia virus that certain groups, e.g. those at cardiac risk, may be considered at a potential greater risk of developing newly appreciated adverse reactions (pericarditis/ myocarditis), and should be excluded from vaccination.

It has been shown that recombinant Ad vaccines can stimulate high level antigen-specific CD8+ T cell responses while recombinant MVA (or ALVAC) vaccines may be particularly suitable to stimulate CD4+ T cell responses. Therefore, MVA vaccines and recombinant Ad vaccines could be complementary when used in prime-boost regimens. This raised the question of what was required regarding pre-clinical testing when two different vectors were used to induce protective immunity. It was considered that, since the vectors in prime-boost regimens were administered at different times, usually with a significant interval between inoculations, independent testing data for each vector were sufficient.

3.2. Recombinant adenovirus vaccines

Besides gene therapy applications, recombinant Ad vectors are being developed as prophylactic vaccines. In particular, an Ad5-HIV gag (subtype B) vaccine is being evaluated in a phase I clinical trial involving a 3-dose regimen in 800 healthy volunteers. Regarding local tolerance of the vaccine, pain/ tenderness, swelling and/or bruising were experienced in 39% of vaccinees. Persistence at the injection site was up to 6 months, which is much longer than the 3-4 days for MVA or ALVAC vectors. Although Flu-like symptoms were experienced by some volunteers; serious adverse events (SAEs) probably related to the vector were recorded in only 6 volunteers (<1%). To establish a more complete safety profile, volunteers are issued with an ID and vaccine report card and required to attend a safety laboratory where tests to assess vector shedding (tonsillar swabs), integration, tumorigenicity and vaccine-induced anti-HIV gag positivity are carried out. About 30% of the volunteers seroconverted by 6 months post-vaccination. Volunteers will need to present their ID card to hospital staff should they require medical treatment in hospital so that they are not confused with HIV-1 seropositive individuals.

3.3. Conclusions: Clinical trial data

- Safety has to be shown in the intended target population; consideration should be given to other populations that may be at risk.
- Shedding study in humans should be done which may then suffice for a given vector backbone with a given insert. Such a study may not have to be repeated for other vaccines using the same vector encompassing other inserts if the given platform can be extrapolated because of the amount of data collected and the comparability of the target population.
- Similar conclusions may be drawn concerning certain animal toxicity studies.
- No significant concerns should result from studies in the clinic, otherwise additional studies or modifications may have to be necessary.
- Comparison of protective immune correlates and the level of those, e.g. antibodies, with a previous marketed, comparable vaccine may be useful with a view to assessing protective efficacy.
- Assessment of dose response in humans is important, but should be addressed on a case by case basis.
- Potential for recombination could also be assessed in clinical trials for those vectors with integration potential.
- Testing of all immune functions is state of the art; if considered relevant for protection, test:
    - humoral responses (antibodies, functional antibodies),
    - cellular responses (CTL, Th) and
    - innate immune responses.
- Identification of correlate of protection should be attempted when feasible.
- Assessment of pre-existing immunity or anti-vector immunity with repeated dosing is important.
- Prime-boost regimens: Consideration should be given to show immunogenicity and safety in humans for each vaccine alone and for the intended prime-boost regimen depending on the antigen used, the route of administration and other issues.
- General issue not specific for vectored vaccines, but generally relevant for prime-boost regimens using 2 different vaccines:
  Even if both vaccines have been characterized separately, animal studies should be done to show that the intended boosting of immune reactions can be achieved *in vivo* and that no synergistic mechanisms compromising safety are occurring, if such mechanisms are expected or suspected, respectively, to occur in animals.

4. Regulatory issues for live viral-vectored vaccines

(K. Cichutek, PEI, Germany, P. Krause, CBER/FDA, US; Dr T Zhou, NICPBP, China; Dr M. Baca-Estrada, Health Canada, Canada)

4.1. EU position

It was noted that no regulatory guidance document specific to the technical requirements for licensure of these novel vaccines was universally available. However, the European Union (EU) Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Products (3), although aimed at gene transfer products for gene therapy, was noted to encapsulate certain principles applicable to vectored vaccines. Vaccines transferring and expressing antigen genes would also be subject to the requirements of Annex I, Part IV, of EU Directive 2001/83/EC, as amended by Directive 2003/63/EC, ("Legally required testing provisions for advanced therapy medicinal product"), which defines gene transfer products as "products resulting from a set of processes aimed at the transfer, to be performed either *in vivo* or *ex vivo*, of a prophylactic, diagnostic or therapeutic gene, i.e., a piece of DNA, to human cells and its subsequent expression *in vivo"*. It was recommended that any proposed guidance on the quality assurance of vaccines based on viral vectors and on the execution of clinical studies should be specific to particular vector categories, e.g. chimeric live viral vaccines, alphavirus-replicon DNA vaccines, modified/attenuated poxvirus vaccines, replication-restricted adenoviral vector vaccines, etc.

4.2. Considerations at CBER, FDA

The issues associated with live-virus vectored vaccines appear to parallel those for other live virus vaccines, with a few additional considerations. Safety considerations and regulatory issues for licensure of live viral-vectored vaccines in the **USA** are therefore considered in relation to relevant examples, including a live-attenuated influenza vaccine (licensed in 2003). These include target disease epidemiology, safety, efficacy, consistency of clinical lots and concomitant administration and should be related to clinical phases of vaccine development: phase I, proof of safety at escalating doses; phase II, proof of concept, immunogenicity and additional safety assurance; phase III, proof of efficacy, immunogenicity, further assurance of consistency and safety; phase IV, safety monitoring, plus post-marketing commitments. With regard to disease epidemiology, vaccine licensure must be based on benefit/risk analysis, for which the likely impact of the vaccine on disease is an important component. However, safety is the primary consideration with a particular need to establish a safe dose range, especially as prophylactic vaccines are administered to healthy subjects. Pre-licensure safety databases, with data from greater than 10,000 volunteer vaccines, are required and can be augmented in phase IV. Efficacy should be demonstrated in trials that permit the establishment of an efficacious or immunogenic dose range. Immunogenicity is most valuable when it can be correlated with protection and is a useful endpoint for

determining consistency. The duration of efficacy should be monitored for many years post-vaccination. Consistency should be proven for at least three consistency lots with respect to consistency of manufacturing parameters, immunogenicity and safety endpoints. With regard to concomitant administration, there is a need to show absence of interference (both immunogenicity and safety endpoints) between existing vaccines and the new vaccine. Appropriate studies to demonstrate this will depend on target age-range for the new vaccine.

Viral replication and potential for reversion from avirulence are major safety issues for evaluating benefit - risk profiles for live virus vaccines. These should be assessed in respect to virus shedding and transmissibility, virus persistence/latency and potential for establishment of chronic infections, immunocompromised and other special at-risk populations, e.g. asthmatics for live-attenuated influenza vaccine, and the availability of effective antiviral agents. Virus shedding studies should, where possible, include appropriate tests to detect virulent revertants. An attendant problem of virus shedding is for blood donations following vaccination with live virus vaccines. Blood products are often administered to immunocompromised patients and therefore it is desirable to establish a period from the day of vaccination in which blood donations should be deferred.

Additional considerations for live virus-vectored vaccines include i) potential for recombination with circulating viruses, ii) effect of prior infection with viruses related to the vector and iii) immunogenicity of subsequent doses, including vaccines against different pathogens that use the same vector backbone. Recombination of a live virus-vectored vaccine with a circulating or reactivated latent virus could theoretically generate a more pathogenic strain. This would be less of an issue for vectors that share little homology with circulating/latent viruses. The risk of recombination should be studied if possible in a non-clinical model system, but should also be considered in clinical study designs. The use of vectors unrelated to those viruses commonly known to cause infections to which corresponding immunity is developed is seen to be advantageous, since immunogenicity of the vaccine should not be compromised. Where prior immunity to the vector is found, higher doses of vectored vaccine could be effective, but might increase overall safety concerns. Alternatively, approaches using priming with a DNA vaccine or other prime-boost strategies may work to overcome prior immunity.

4.3. Issues considered in China

Regulatory authorities (SFDA) have made provisions for the safe administration of genetically-engineered products by issuing technical guidelines for gene transfer products (for gene therapy applications), DNA vaccines, recombinant DNA products and, more recently, prophylactic live virus-vectored vaccines (March 2003). These requirements include sequence analysis of gene constructs, tests for genetic stability, characterization of recombinant viruses and cell banks for vaccine propagation, characterization of expressed heterologous antigens, tests for identity and purity of vectored vaccine, tests for extraneous agents and process residues, and thorough toxicological and

immunological evaluation. Non-clinical testing also should include tests to show immunogenicity, i.e. development of humoral and cell-mediated immunity and, where possible, protection against the pathogen in animal model systems. The effects of any adjuvants should be analysed. However, the diversity of such products gives rise to the need for a case-by-case, science-based treatment of individual products.

Currently, in China about 40 manufacturers are involved in vaccine production. At least thirty-seven prophylactic vaccines (viral, bacterial, subunit, recombinant, combined, etc.) targeted against 26 diseases are being manufactured. Live virus-vectored vaccines based on poxviruses, adenovirus, retrovirus, herpes simplex virus and vesicular stomatitis virus are being studied. Data from these studies should provide valuable information and lead to safer and more efficacious virus-vectored vaccines in the future.

4.4. Health Canada position

Requirements for clinical trial application and marketing authorization of live viral vectors in Canada are similar to those in the US and Europe. At the stage of phase I clinical trials adequate pre-clinical safety and immunogenicity/protection (if appropriate animal model system available) data together with satisfactory quality assurance specifications of clinical lots are required. At phase II, in addition to immunogenicity, further safety data relating to the clinical use of the vaccine, including information on the risk of persistence, shedding and transmission, are required. Canadian *Food and Drug Regulations* defines activities for which GMP compliance is to be demonstrated prior to the issuance of an establishment license. Alternative means of complying with GMP Regulations can be considered with the appropriate scientific justification and at different stages of development (Phase I, II, III). Different approaches may be taken as new technologies emerge. Health Canada provides further guidance, specific documents can be found in Health Canada web site (http://hc-sc.gc.ca/). For vaccines based on viral vectors fully validated QC tests should include those applied to cell substrates, e.g. tests to demonstrate freedom from extraneous agents, to quantify residual cell DNA and protein, to assess stability of packaging cell lines, to detect replication competent viruses, etc. Genetic stability is crucial and equally applicable to the vector backbone and the ligated antigen-encoding construct. The use of monoclonal antibodies against major epitopes in the expressed antigen may help to monitor for genetic changes in the transgene. It will be important to check for any changes in tropism between the parental virus and the recombinant virus as these could lead to greater virulence, as could also mutations that undermine attenuation. For certain vectors, e.g. YFV-based vectors, increased virulence may be detected in appropriate animal model systems, e.g. neurovirulence tests in mice or monkeys. Demonstration of compatibility with other vaccines is also important. Pre-existing immunity to the vector should be assessed as this could significantly reduce efficacy.

4.5. Conclusion: Specific regulatory issues related to live virus-vectored vaccines

Appropriate potency assays with relevant and well-characterized reference materials should if possible be carried out; these may differ to those used for conventional vaccines. Particle counts or some other physical measure of virus concentration could also be important for many vaccines. Such measurements would provide a minimum specification for safety and efficacy and also assure consistency among batches. For example, chimeric YFV might be assessed on the basis of particles per dose. A characterization test used in phase I/II clinical trials could subsequently provide data for a release parameter specification. Specific tests such as determination of the ratio of antigen expressing particles; the proportion of empty particles and monitoring the ratio of infecting/expressing cells may be required for licensing of vaccines based on live, viral vectors.

An example of a recombinant live virus-vectored vaccine expressing up to 5 different antigens was raised with regard to the stringency of results for lot release. Are expression levels for all 5 antigens required? It was considered premature for this to be tested ahead of phase I; the "right" level of antigen expression should be critically appraised at phase III together with selection of the most appropriate potency tests and specifications.

The Consultation agreed that only if virus-vectored vaccines were neurotropic should neurovirulence tests be used for lot release. Neurovirulence tests would be useful if they indicated a difference between batches or provided evidence batches can change from one to another. Batch-to-batch testing of such vaccines by testing for specific mutations associated with reversion was also considered to be useful. However, alternative testing would be required for detecting replication competent vectors/viruses derived by recombination or rescue by wild type viruses. Manufacturing strategies should prevent the generation of replication competent viruses, e.g. replication competent Ads (RCA) should not be formed if the replication-restricted Ad-vectored vaccine is propagated in cell lines like PER.C6 as recombination with integrated complementing E1 sequences cannot occur. Nevertheless, the risk of recombination/rescue may be increased *in vivo* and therefore appropriate tests to monitor vaccinees for the presence and/or shedding of replication competent viruses should be developed.

5. Recommendations to WHO and priorities for future work

The consultative group agreed that guidelines on production and control, nonclinical and clinical testing of vaccines based on viral vectors will be a useful tool since there are no globally accepted guidelines for preventive vectored vaccines, at present. However, it was recognized that some points are already covered in the existing guidelines published by WHO and some international bodies such as EMEA (5,3). Technical requirements and clinical trial approval processes are in place in some countries and national guidelines are being developed in some countries (EU, USA, Canada and China).

The Consultation recommended to WHO to publish a detailed meeting report which will provide points considered at the meeting and concerns raised by vaccine developers and regulators and will serve as a first step towards the development of the WHO guidelines.

The following specific concerns for vaccines based on viral vectors were identified as issues of critical importance to be investigated further:

1. Potential of recombination with wild type pathogenic strains
    i. Vector – circulating virus could create a more pathogenic strain.
    ii. This issue should be addressed *in vitro* or in animal studies.
2. Implications of prior infections on the immunogenicity of vectored vaccines..
    i. Prior infection with related viruses may reduce vaccine immunogenicity (e.g., adenoviruses, poxviruses (smallpox vaccine))
    ii. Immunogenicity of subsequent doses, especially with different gene in same vector (e.g., modified poxviruses, adenoviruses): should be addressed if relevant
3. Genetic stability of replicating viruses *in vivo* should be studied focusing on:
    i. The sequence insert, and known areas of attenuation
    ii. Known epitopes
4. Potential changes of tropism may lead to new properties of replicating viruses and should be carefully evaluated.
5. Tests for absence of reversion to virulence should be performed when an attenuated vector is used.
6. The absence of replication competent virus when replication incompetent vectors are used should be demonstrated.
7. Public acceptance of vectored vaccines with specific safety concerns could be an issue. A need for a forum to discuss concerns, and how best to communicate the risks and benefits of the new approach to general public was identified and WHO was requested to take a lead on it.

The Consultative group recommended to WHO to present this meeting report to the Expert Committee on Biological Standardization at its meeting in 2004 and to obtain advice on the way forward.

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