

Short communication

Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, WHO, Geneva, 2–3 September, 2004[☆]

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Abstract

The World Health Organization (WHO) is undertaking consultations on immunological responses as parameters for evaluation and licensure of new Japanese encephalitis (JE) vaccines. Immunological markers could be used by vaccine developers and regulatory authorities to assess vaccine efficacy in absence of clinical efficacy data. The consultation which is reported here reviewed current data on mechanisms of protective immunity gathered from animal experimentation, clinical data from licensed vaccines and from vaccine candidates still in clinical development. Immunological assays and readouts for use in evaluation of candidate vaccines were also discussed. The consultation made a series of recommendations for specifications on immunological criteria to assess JE vaccine efficacy. More detailed recommendations will be drafted following further consultations to serve as WHO guidelines for evaluation and licensure for new JE vaccines.

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1. Introduction and objectives

Japanese encephalitis (JE) is an acute viral infection of the central nervous system. The disease is caused by JE virus, which is a member of the genus *Flavivirus*, and is endemic throughout large parts of East and South East Asia. The virus is transmitted by *Culex* sp. mosquitoes and it has recently shown considerable geographical spread into areas of Asia previously unaffected. The severity of the disease and its spread has put prevention and control of JE high onto the

public health agenda of affected countries in Asia. While an internationally registered formalin-inactivated JE vaccine exists, it is relatively costly, requires several doses in the primary series and repeated booster doses, hereby making it less suitable for widespread immunization in the disease endemic populations. Hence, there remains the need for a safe, inexpensive and efficacious JE vaccine suitable for use in disease-endemic countries [1].

In their introductory remarks Drs. Joachim Hombach (WHO) and Ichiro Kurane (National Institute of Health, Japan) reminded the participants that several promising alternative vaccines exist, but these are either not registered for international use, or are still in clinical development. The clinical evaluation of new JE vaccines for efficacy is complicated by the low incidence of JE and the availability of an internationally registered product. Under these circumstances, a placebo controlled trial would be difficult to justify on ethical grounds, and a comparative trial between licensed

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and new vaccine using the endpoint of prevention of clinical illness would require unpractically large sample sizes. Hence, there is a need to determine a vaccine-induced immunological readout that may be used as a surrogate for protection from disease. The availability of a universally accepted surrogate would promote clinical development and evaluation of new vaccines and thereby contribute to the accelerated introduction into immunization programmes.

The WHO consultation on immunological endpoints for Japanese encephalitis vaccine trials was organized to: (i) review current scientific evidence on immunological mechanisms conferring protection against JE; (ii) discuss possible regulatory expectations for registration of a vaccine in absence of clinical efficacy data; and (iii) review assays and methods suitable for measuring immune effector mechanisms correlating with protection. Based on these considerations, recommendations for specific immunological criteria were developed, which are presented in this paper.

The process for the development and use of WHO recommendations and guidelines to national regulatory authorities (NRA) on evaluation and registration of biologicals was presented and discussed by Dr. David Wood (WHO). Although not binding, these documents provide guidance to NRAs and help to set common standards. The development of recommendations and guidelines starts with stakeholder consultations, such as the one presented in this report. Recommendations are developed and further discussed with the scientific community. When consensus is achieved, they are submitted to the WHO Expert Committee on Biological standardization (ECBS), which decides on adoption. Adopted guidance on regulatory expectations is published in the WHO Technical Report Series [2].

2. Immune mechanisms related to disease protection

The session on immune mechanisms related to protection from disease was introduced by a presentation from Dr. Scott Halstead (Uniformed Services University, USA), who discussed flavivirus challenge data in animals and humans, and also drew lessons from other infectious diseases. He described how challenge studies with dengue virus in primates were used to define protective efficacy against dengue wild type (WT) inoculations. Taking the example of measles, antibody titres following natural infection were compared with those induced by vaccination, demonstrating how a virus' antigenic characteristic may be changed during the attenuation process that produces the vaccine strain [3]. Measles WT infection gives hemagglutination inhibition (HI) and neutralizing (NT) antibodies for life. In contrast, after vaccination HI antibodies wane, but NT antibodies are retained in most subjects, though not at very high titres. Despite dropping titres protection is maintained. Even after repeat vaccinations, HI and NT antibody levels drop to a baseline resting level. Similarly, with polio vaccine NT antibodies wane over several years, despite repeat vaccinations;

the interval since the last vaccination being critical to how low antibody levels drop. In contrast, with the live attenuated JE vaccine SA 14-14-2, repeat vaccination leads to increased antibody titres [4]. Dr. Halstead concluded that through attenuation of virus, immune responses to vaccine viruses may be different to the responses following natural infection. Also, antibody titres are not necessarily a good marker of individual protection, because individuals are still protected despite low or waning antibody titres. Given that each live attenuated virus is unique biologically, defining immunological correlates is complex.

The following presentations addressed evidence, both from clinical and preclinical studies, for antibody and T cell mediated effector function in protection against JE.

Dr. Ichiro Kurane addressed protection against JE by passive transfer of immune sera. Theoretically, antibodies can protect against infection by neutralizing virus when released after a mosquito bite, and can attenuate disease by limiting viraemia and CNS spread. Oya et al. inoculated mice with sera at various dilutions to determine the amount of antibodies required for protection [5]. These studies suggested that a neutralizing antibody titre of 1:10 is protective. Lubiniecki [6] examined protective immunity in weanling mice of vaccinated pregnant mice, as a natural passive transfer experiment and showed a linear relationship between levels of naturally acquired maternal antibody and protection. Progeny of immunized mothers did not manifest an immunological response post challenge, likely to be due to sterilizing neutralizing antibody. Konishi performed passive transfer of various levels of antibody in mice. Monoclonal antibody experiments showed that antibodies with the highest neutralizing activity conveyed the highest levels of protection. However, in a separate study, primed animals with no detectable NT antibody still were protected against challenge [7]. In summary, passively transferred antibody can protect mice from infection in a dose-dependent manner, and can contribute to recovery from infection. However, even in the absence of pre-existing NT antibody, protection of mice from lethal challenge can be achieved if the immune system is primed to mount an efficient anamnestic immune response.

Additional aspects of antibody-mediated protection were discussed by Dr. Tom Monath (Acambis). He pointed to the wealth of scientific data showing protection through passive transfer of antibody, which resulted in multiple published studies since 1974. Moreover, these studies helped to establish the relationship between antibody titre and protection. A recent study was reviewed, where hyper immune mouse ascitic fluid against the chimeric yellow fever/JE vaccine (ChimeriVax-JE) and formalin inactivated mouse brain vaccines (both belonging to genotype III) was produced, and efficacy of these antibodies in protecting C57BL/6 mice against intracerebral challenge with JEV strains of all four genotypes and different virulence assessed [8]. Protective capacity of antibody was clearly demonstrated, including resistance to challenge with heterologous JE virus genotypes. Protection level depended on titre of antibody, virulence of the

challenge strain, and compatibility of genotype of immunogen and challenge. As previously observed, genotype I virus was protected less well by ascitic fluid raised against genotype III. While passively transferred NT antibodies may act in a somewhat strain-specific way in experimental settings, the question remains whether T cells help to mount broader protective responses after active immunization.

Dr. Sharone Green (University of Massachusetts) spoke on the role of T cells in protection against JE. As a general remark, it is noteworthy that T cell effector function helps to prevent disease, but does not necessarily prevent infection, as exemplified by cytotoxic T lymphocytes (CTL) that attack cells, once they have become infected. However, this mechanism is critical to limit virus production and dissemination in the tissue. T cell studies of JEV infection and JE vaccines were reviewed, as were the expected protective immune responses to other flaviviruses. There is solid evidence that heterologous immunity to flaviviruses is mediated by CTL. Murali-Krishna [9] showed that both CD4 and CD8 T cells were required for protection against intracranial inoculation using passive transfer experiments. More specifically, the role of CD8 T cells was analysed in a CD8^{-/-} mutant mouse using West Nile virus [10]. In CD8^{-/-} mutants there is no difference in viraemia as compared to wild type mice, but CD8^{-/-} mutants are more likely to die, with higher CNS viral burden, suggesting that T cells have a role in controlling and clearing viral spread in the tissue. Studies of CD4 and CD8 T cells may provide useful data for monitoring vaccine efficacy, and T cells might contribute to minimizing viral replication. In fact, some JE virus replication can be observed in vaccines exposed to natural infection that is abrogated by anamnestic T and B cell responses. In conclusion, it appears that although the primary protective mechanisms for current vaccines may be antibody mediated, T cell function can be critical to control JE and clear infection, as well as for mounting efficient anamnestic immune responses. This principle is likely to apply to other flavivirus vaccines as well.

The role of immunological memory in long-term protection was reviewed by Dr. Tom Monath. He presented data on booster studies using JE inactivated vaccine in individuals who previously had received ChimeriVax-JE vaccine [11]. Individuals primed with ChimeriVax-JE displayed typical memory responses. Importantly, there was no difference in memory response irrespective to whether vaccine recipients had measurable antibody levels 9 months after priming, showing that immune memory persists despite waning of antibodies. Dr. Monath suggested that the kinetics of the memory response may be sufficiently fast to prevent neuroinvasion and development of disease, and hence confer protection from disease. In that context a better characterization of memory responses following natural infection would still be desirable. Overall, memory responses to challenge with JE antigens were considered a useful adjunct to assessing vaccine efficacy. It is noteworthy that the demonstration of induction of memory data is a regulatory expectation for licensure of certain new conjugate vaccines to prevent bac-

terial disease, such as meningococcal C and pneumococcal vaccines [12].

Dr. Tom Solomon (University of Liverpool) reviewed data from efficacy trials of JE vaccines. While several JE vaccines are in use today, only formalin-inactivated JE vaccine has been evaluated in placebo-controlled trials. In 1965, a placebo (tetanus toxoid) controlled trial of inactivated unpurified JE vaccine (Nakayama strain) was conducted in Taiwan, and in 1984–1985 a placebo (tetanus toxoid) controlled trial of inactivated, purified JE vaccine of Nakayama strain and bivalent Nakayama/Beijing-1 strains was carried out in Thailand. The Thailand study [13] was discussed in detail. Bivalent and monovalent vaccine showed the same (91%) protective efficacies. There were 13 cases of hospitalized JE, 11 were in the placebo and 2 in the vaccine groups. Also, some protection from dengue was suggested by slightly reduced attack rates and reduced disease severity for that disease in the vaccine groups. It was not possible to define the levels of antibody, as measured by the NT assay, that correlate with clinical protection or the duration of protection in these studies.

Drs. Dianliang Lei (WHO) and Theodore Tsai (Wyeth) discussed clinical data from the live attenuated SA 14-14-2 vaccine that has been studied in two large case control studies. One post-licensure case control study was conducted in Sichuan Province, China [14]. In this study, among children <15 years of age efficacies of 80% (CI 44–93%) for a single-dose regimen and of 97.5% (CI 86–99.6%) for a two-dose regimen, respectively, was estimated. The second study conducted in Nepal in 1999 found a 99.3% efficacy with a single dose regimen. The campaign delivered some 160,000 doses just prior to a disease outbreak, suggesting that the vaccine confers protective efficacy as early as 2 weeks after vaccination [15]. There are several studies showing somewhat variable degree of seroconversion after one dose. One study, conducted in a northern, JE non-endemic Yunnan Province of China, showed persistence of NT antibodies in 88% of recipients after 6 years following a two dose regimen.

In summary, the clinical data of the JE vaccines currently in use provide some evidence for the role of NT antibody in protection, but do not establish a firm correlation between the level of antibodies and clinical protection from disease.

3. Regulatory and public health considerations

Several representatives from different regulatory agencies, including some from disease-endemic countries, provided their personal opinion on the regulatory expectations for the licensure of JE vaccines. The views given do not necessarily represent the official positions of the respective authorities.

Possible expectations by the US FDA from a regulatory dossier for licensure of a new JE vaccine based on surrogate markers for efficacy were discussed by Dr. Lew Markoff (US FDA). He explained that the concept of using surrogates instead of clinical endpoint for licensure of a vaccine was not new and has previously been applied to hepatitis

B and meningococcal type C vaccine. Established surrogate markers also exist for yellow fever and polio vaccines. Examples were given on how these surrogates were derived. Inline with a previous publication on JE vaccine evaluation [16], Dr. Markoff considered that evidence for efficacy might be shown by NT antibody responses and by demonstration of protection in a suitable animal model for an established registered JE vaccine of known clinical efficacy, and then demonstration of equivalence of the new vaccine to this. This could include the following steps: demonstrate protection in the animal model by challenge of animals passively immunized with sera from human vaccines; correlate these results with NT titres; head to head small scale immunogenicity trials using licensed comparator JE vaccine as a control in a JE non-endemic area; finally, larger safety trials of 3000–5000 subjects will be required. While the comparator vaccine would most likely be the licensed inactivated vaccine, there was discussion if more comparator vaccines should be included in countries where more than one vaccine is licensed. There was also discussion on the most appropriate animal model, which could be mouse, hamster or monkey. It was felt that the US FDA animal efficacy rule would not apply to licensure of a new JE vaccine [17].

The concept and design of non-inferiority studies was discussed by Dr. P. Lachenbruch (US FDA). A non-inferiority study compares an investigational product to a reference product with the goal of showing that it is not worse by a pre-specified margin. The reference product or active control arm would have to be included into the study, and historical controls would not be appropriate. A non-inferiority study could compare continuous variables (e.g. GMT) or dichotomous thresholds (e.g. seroconversion), noting that the latter might require up to 30% larger sample sizes. Critical questions remain on the time points of measurements and the appropriate margin of acceptable inferiority (delta). WHO recommends non-inferiority margins of 10% [18]. Finally, it was pointed out that a study design of a three-dose versus a one-dose vaccination regimen, which would be likely if inactivated and live vaccine were compared, would limit comparability of some qualitative immune response measurements, such as heterologous protection.

Experiences from countries with licensed JE vaccine were presented in a subsequent session. Dr. Yupin Lawanprasert (FDA Thailand) provided a regulatory perspective from a disease-endemic country that locally produces inactivated vaccine, yet is interested in hosting clinical evaluation of new vaccines. Of note, in Thailand, every lot of locally produced vaccine is tested in humans for immunogenicity and adverse events, in a comparison with imported formalin-inactivated vaccine. Dr. Jin-Ho Shin (WHO) discussed experiences with licensure of SA 14-14-2 vaccine by the South Korean FDA. Registration of the vaccine, imported from China, started in 1997 and final authorization was granted in 2002. WHO guidelines that had been specifically developed for live attenuated JE vaccines helped to clarify regulatory concerns. For licensure, a small scale clinical study has been conducted in

South Korea [19] and a post-marketing surveillance scheme established. Dr. Dianliang Lei (WHO) on behalf of Dr. X Yu and Dr. GM Dong of the National Institute for the Control of Pharmacological and Biological Products, Beijing, China, reviewed the development of SAM-14-2 vaccine in China. There is a wealth of immunogenicity, efficacy and safety data, much of which has not been widely publicized outside of China. The regulatory perspective from Australia was given by Dr. Grahame Dickson (Therapeutic Goods Administration TGA, Australia). TGA follows closely EMEA guidelines CPMP/EWP/463/97 on new vaccines [20]. Australia hosts the clinical development of the ChimeriVax-JE vaccine. TGA considers accepting pivotal immunogenicity data and a non-inferiority trial design. One particular concern was that vaccines currently available and in development are genotype III vaccines, whereas the genotypes currently endemic in Australia are different. In Australia, exposure to other flaviviruses has the potential to complicate the immune response. Post marketing surveillance should also look at the need for booster vaccination.

Dr. Robert Breiman (KEMRI, Kenya) discussed public health expectations from JE vaccine efficacy trials, by drawing on lessons from Hib, Rotavirus, pneumococcal and typhoid vaccines. If a vaccine is licensed through a simplified process, such as non-inferiority trials measuring immunological endpoints, two aspects need particular scrutiny: vaccine safety and public health impact of vaccination. The rotavirus vaccine experience (where a 1:5000–15000 risk of intussusception was reported shortly after licensure and wide scale vaccination of infants) showed it is important to have substantial safety information before licensure, combined with adequate country-specific data on cost and impact of disease defined in settings with different endemicity. On that basis identified risks can be balanced with potential individual and public health benefit before making decisions about continued use of the vaccine.

Prior to introduction of a new, effective vaccine, education programmes should be designed so that expectations regarding the value of the vaccine are appropriate. Efforts will be needed to make it clear to policy makers and the public that while the introduced vaccine will prevent most cases of JE, it will not prevent other cases of encephalitis which may also be endemic or occur in epidemics or sporadically. Early adoption and incorporation into EPI vaccination schedules represent another important factor for rapid introduction of a new vaccine. Post-marketing studies will be important to identify possible rare adverse events, and if co-administration with measles is aimed at, studies should be designed with and without measles vaccine to ensure there is no interference or increased adverse outcome.

4. Assays and methods

Dr. Jane Cardoso (University of Sarawak, Malaysia) opened the discussion on immunological assays and methods

by addressing fundamental questions on plaque reduction neutralization (PRNT) assays and the comparability of results. PRNT is the most commonly accepted test to measure functional antibody able to inactivate virus, and a titre of 1:10 is regarded as the minimum protective level [5]. PRNT is a demanding functional assay and laboratories are doing the test according to varying protocols. Protocols vary regarding cells substrate, incubation conditions, addition of complement, size of wells and percentage reduction of end-points (50% is preferred). Test results between laboratories are therefore not readily comparable. In order to achieve lab-to-lab comparability, tests need either to be standardized or a calibrated international reference serum needs to be developed. A collaborative study, organized by WHO and NIBSC, is currently ongoing to define sera suitable to be calibrated into international units. This study was presented and discussed by Dr. Morag Ferguson (NIBSC, UK). There was discussion on the origin of serum, which should be reactive with different JE virus genotypes, favouring sera from naturally immune individuals.

Discussing findings from her laboratory, Dr. Jane Cardosa found responses to immunization dependent on previous or concurrent asymptomatic infection with WT JE virus. For a group of children 1 month after second dose of inactivated vaccine only 27% had positive PRNT50, but 90% were positive at 1 year, coincidental with asymptomatic infection in the interval, as determined by measuring antibodies to NS1 during the intervening period. In contrast to PRNT, IgM seroconversion is readily seen after first and second dose, indicating a possible role for Mac or GAC ELISA as an initial screen for antibodies. In summary, higher PRNT50 titres are seen in individuals with evidence of an anamnestic response, and in particular in individuals with a history of natural infection. Antibodies to prM may be useful in distinguishing flavivirus exposure, and antibodies to NS1 are useful in determining prior infection, as distinguished from prior vaccination with an inactivated vaccine.

Dr. Sutee Yoksan (Mahidol University, Thailand) discussed the evaluation of children immunized with inactivated mouse brain derived JE vaccine in Thailand. Here, genotypes I, II and III have circulated over the last few decades, but all 13 recent isolates were genotype I. Vaccination of 60 children with Nakayama or Beijing-1 inactivated vaccines (both of genotype III) gave very good seroconversion to homologous virus, but lower titres to heterologous virus of different genotypes, following the primary immunization of two doses. However, after the booster dose at 1 year there was good heterologous reactivity as well. This finding leaves open the question on the relevance of low heterologous titres, as measured in PRNT, and epidemiologic evidence does not support lack of protection to heterologous strains during this period. Similarly, no epidemiologic evidence exists that YF vaccine could have reduced efficacy against different wild-type strains.

Methods and approaches for assessing T cell effector function in vaccine recipients were reviewed by Dr. Sharone

Green. In practical terms, complexity and costs of the tests, timing of samples, and volumes of blood were discussed. Cell-specific and cruder, cell population based, assays are available. The fundamental question remains whether T cell assays can functionally be linked to protection. Although cross-genotype lymphoproliferation, as it has been documented in a WHO sponsored study, may not be proof for protection, it suggests that JE vaccine induced memory T cell responses help in generating anamnestic responses of broader specificity which appear to be associated with post-challenge protection [Sharone Green, personal communication]. During the discussion, it was concluded that understanding the protective role of JE virus-specific T cells in JE infection remains an important research question, and that the characterization of T cell responses from individuals who recovered from natural infection should be pursued. Based on current knowledge, T cell data remain supportive to antibody data.

Finally, some research needs and strategies for improved functional assays were discussed by Dr. Jane Cardosa. As a prelude to vaccine studies there is a need for high-throughput user-friendly screening tests to determine flavivirus-immune status of potential study populations. MAC and GAC ELISA for NS1 antibodies were proposed to distinguish between yellow fever, JE and dengue infection.

5. Recommendations

Dr. David Wood opened the discussion on recommendations for specifications to assess JE vaccine efficacy by outlining the process WHO applies to establish guidelines and recommendations. Subsequent to the publication of the meeting proceedings, a small drafting group will be constituted that develops guidelines based on the meeting conclusions. More stakeholder consultations will be conducted, if needed. Draft guidelines will be circulated to experts, and subsequently submitted to ECBS. Publication of adopted guidelines will be done as addendum to the WHO guidelines for production and quality control of live attenuated JE vaccines and inactivated JE vaccines [21–22]. In principle, the guidance should be applicable to all new JE vaccines, including vectored vaccine candidates, and eventual vaccine-specific criteria should be flagged out.

Concluding from the discussions of the meeting, the following recommendations were made during a closed session attended by WHO temporary advisers.

5.1. Scientific rationale for the selection of correlates for protection

NT antibodies provide the best evidence that protective immunity has been established, and the functional assay of neutralization shows correlation with protection, as demonstrated in multiple passive transfer studies in animals. A linear titre-protection relationship exists, and data from efficacy trials corroborate the role of NT antibodies in protection.

Moreover, NT antibody mediated protection has been demonstrated for homotypic and also for heterotypic challenge. On the other hand, low antibody titres do not exclude protection.

5.2. Primary endpoint

A quantitative analysis of NT antibodies should be done in a head-to-head comparison with a registered vaccine, following a non-inferiority trial design. A reasonable threshold antibody level for protection is a 1:10 dilution in a 50% PRNT.

For comparability of assays, broadly JE virus genotype cross-reactive sera should be calibrated in terms of international units, and appropriate negative controls be used. To further improve comparability of results, guidance on validation of in-house PRNT assays should be provided, as well as fundamentally important aspects of the assay be described. Guidance should also be established on the time of serum collection after a primary series of vaccination, with 4–6 weeks after last dose as reference.

For head-to-head comparison, the comparator vaccine should be any licensed vaccine, acceptable to the regulatory agency of the country where the vaccine is being tested; the regulatory authority may want to look carefully at the dosing schedule of the comparator vaccine to ensure it is appropriate. If vaccination cannot be blinded, blind evaluation of sera should be assured as a minimum. Careful consideration should be given to the selection of the virus strain used for neutralization in PRNT assays.

Non-inferiority should be measured as percentage of seroconversion, but the provision of GMT data is encouraged. The threshold of 1:10 is considered reasonable, and measurements of lower thresholds could only be considered if a reliable discrimination between positive and negative samples was possible. As predefined margins in the 95% confidence interval, for the proportion of responders a margin of not more than 0.1 appears acceptable. This means that the lower bound of the 95% confidence interval for $p_{\text{new}} - p_{\text{old}}$ must be greater than -0.10 . For the comparison of titres the ratio of GMT new: comparator vaccine should exceed 0.5. In each of these measures it may be appropriate to expect the comparator vaccine to attain a given level. For example, seroconversion proportion might be required to be at least 75% in the comparator group. Similarly a standard could be imposed on the GMT for the comparator group.

5.3. Additional information to support registration

Additional information is helpful to support the regulatory dossier. As these data can be vaccine type-specific, they do not need to be derived from a head-to-head comparison with a licensed vaccine. Supportive elements could include data on immunological memory, a qualitative analysis of neutralizing antibodies, passive protection studies in animal models, and the demonstration of T cell responses to the vaccine.

Immunological memory and the demonstration of anamnestic immune responses can be an important element in

support of vaccine efficacy. Administration of a booster dose of vaccine antigens at the normal dose, which might typically be at 6 months to 1 year after completion of the primary vaccination, with measurement of antibody approximately 1 week later, may be useful to demonstrate immunological memory, in particular if antibody after primary round of immunization is waning. The use of an inactivated vaccine to elicit an anamnestic or booster response in humans is recommended to avoid the dampening effect that pre-existing antibodies might have if a live vaccine was administered as booster. These studies can potentially be conducted in a post-marketing setting.

Qualitative analysis on NT antibodies relates to their capacity to neutralize all four JE virus genotypes. As there is no conclusive data suggesting that reduced cross-neutralization capacity relates to decreased protection, such data require careful analysis. Also, it is important to note that NT antibody responses are broadened by repeat immunizations.

Passive protection in animal models by transfer of human antibodies and subsequent challenge provides additional evidence for functional activity of antibody. Guidance on the choice of the appropriate animal model and experimental design should be provided. Challenge virus should include homologous and heterologous virus at standard dose, e.g. 100 LD₅₀, taking into consideration the different levels on neurovirulence virus strains can show.

Data on T cell responses should be considered as supportive experimental data. Such studies could include evaluation of both T cell effector function at early time points following vaccination, as well as in relation to evaluation of long-term immunological memory. Guidance on validation of in-house standardized methods would be necessary to accomplish such studies.

6. Concluding remarks

It was not the aim of the consultation reported in this document to discuss vaccine safety. Every dossier submitted for registration should comprise the necessary data on vaccine safety, and measures should be put in place to assure appropriate post-marketing surveillance. Bridging studies on vaccine efficacy and safety might be needed if the vaccine is used in populations different from those where the vaccine was initially evaluated, in particular if at risk populations, such as HIV-positive or malnourished children are targeted.

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